EVOLUTION

INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

PUBLISHED BY

THE SOCIETY FOR THE STUDY OF EVOLUTION

Vol. II **MARCH, 1948** **No. 1**

HYBRIDIZATION OF THE HABITAT

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Received November 7, 1947

It has been the experience of most biologists that hybridization between species is rare in nature. Many biologists encounter interspecific hybrids in the field so rarely as to doubt if they really occur there at all or else find them under special circumstances (Epling, 1947) which raise serious doubts as to the importance of hybridization under natural conditions. After a series of investigations (Anderson, 1936b, 1936d; Anderson and Hubricht, 1938; Anderson and Turrill, 1938) it has been my own experience that clear-cut out-and-out hybrids are seldom met with, even when a deliberate search is made for them, and that hybrid swarms of bizarre recombinations are found, if at all, only under peculiar circumstances.

It was once the common opinion (Zirkle, 1935) that this lack of evident hybridization was caused by the sterility of interspecific hybrids. Experimental evidence has not confirmed this judgment and modern advocates of reproductive isolation (Mayr, 1940) as a species criterion have had to phrase their definitions to permit semi-fertility between distinct species. For the higher plants there is an impressive amount of experimental evidence on this point though it is widely scattered. There are the papers of the early hybridizers (Focke, 1881; Roberts, 1929; Zirkle, 1935), much work in genetics (see for instance East, 1913, 1916) and experimental taxonomy (Clausen, Keck, and Hiesey, 1946), and the experience of numerous plant breeders. The latter, by far the largest of these three bodies of evidence, is not too accessible to most scientists since it has to be dug out piecemeal from such compendia as Rehder's Manual of Trees and Shrubs (1940). A critical summary of all this evidence is badly needed. Lacking one, the evidence for the higher plants may be roughly summarized as follows: Well-differentiated species of the same genus may or may not be interfertile when tested experimentally. On the whole it seems to vary with the genus. There are certain genera in which interspecific hybrids are difficult to make and are sterile. There are others, equally exceptional, in which the widest possible crosses within the genus will yield fertile or semi-fertile hybrids (Anderson and Schafer, 1931). The yellow trumpet Narcissi are so completely fertile with the flat, white-flowered Poets' Narcissi that the whole business of supplying new garden hybrids has been founded on it (Calvert, 1929). The Poets' Narcissi (in themselves a whole group of species and sub-species) are so interfertile with the species complex making up the long-crowned yellow daffodils that it has been possible to accomplish

such recombinations as the transfer of the deep red-orange pigment from the rim of the tiny central eye in a Poets' Narcissus to the brilliant flaring orange trumpet of such modern "red trumpets" as the variety "Fortune" (Anderson and Hornback, 1946). The commonest condition among the higher plants seems to be that in which crosses within a species group are easy to make while intergroup crosses are difficult or impossible.

If, therefore, we base our explanation of the rarity of hybrids under natural conditions on the experimental facts we can make the following summary: 1. In a minority of the cases where related species occur near each other, they are completely intersterile. 2. In certain cases hybrids can be formed but are sterile. 3. In a surprising number of cases the experimental evidence shows that the species can be crossed readily, in the breeding plot, but no hybrids appear under natural conditions. 4. In many cases which have been carefully investigated, hybrids, though usually rare, do occasionally occur but leave no apparent descendants except under unusual conditions.

This experimental evidence, such as it is, justifies the generalization that species maintain themselves as recognizable units even where they are interfertile and even when there is a considerable opportunity for them to hybridize. Why should this be so? Before we can answer the question we must first have clearly in mind the known results of such hybridization under experimental conditions. If we ignore the complications due to polyploidy and other important but more-or-less specialized phenomena, the usual results of hybridization are readily summarized. They were well established by the early hybridizers and have been abundantly confirmed by modern genetic research.

1. The first hybrid generation is intermediate between the parents and is as uniform as they are, or even more so. It is usually more vigorous than either and more robust in nature.

2. The second hybrid generation, while on the average intermediate, is extremely variable. Usually no two individuals are alike. The variation is usually bewildering, but it can be shown to have a general trend from a few individuals more or less like one of the parental species, to a great bulk more or less like the first generation hybrid, to a very few more or less like the other parental species.

3. The facts with regard to the backcrosses are equally well established but unfortunately have been so little stressed in modern times that they are not as generally familiar to biologists. If the first hybrid generation is crossed back to either parent, the first backcross is made up largely of plants which resemble the species to which they have been backcrossed. If we take one of these and cross it back a second time to the same parent, not only will the seedlings resemble this species very much indeed but some of them may be nearly or quite indistinguishable from it. In all the cases with which I am personally familiar, many, if not most, of the first and second back-crosses, if found in nature, would by taxonomists be accepted as varieties or slightly aberrant individuals of the species to which they were back-crossed (see fig. 1). Few taxonomists, even those specializing in that group of plants, would even suspect that many of these back-crosses were of partially hybrid ancestry. For the genus Apocynum we have a detailed experimental record of back-cross morphology and the taxonomic reaction to it (Anderson, 1936b).

In the genus Tradescantia, by rigidly experimental methods (Anderson, 1936a, 1936d; Anderson and Sax, 1936; Anderson and Woodson, 1935), including the production of experimental backcrosses, it has been possible to demonstrate that the principal result of hybridization, in those cases where it did occur, was a series of such backcrosses to one or to both parents. To this phenomenon I gave the

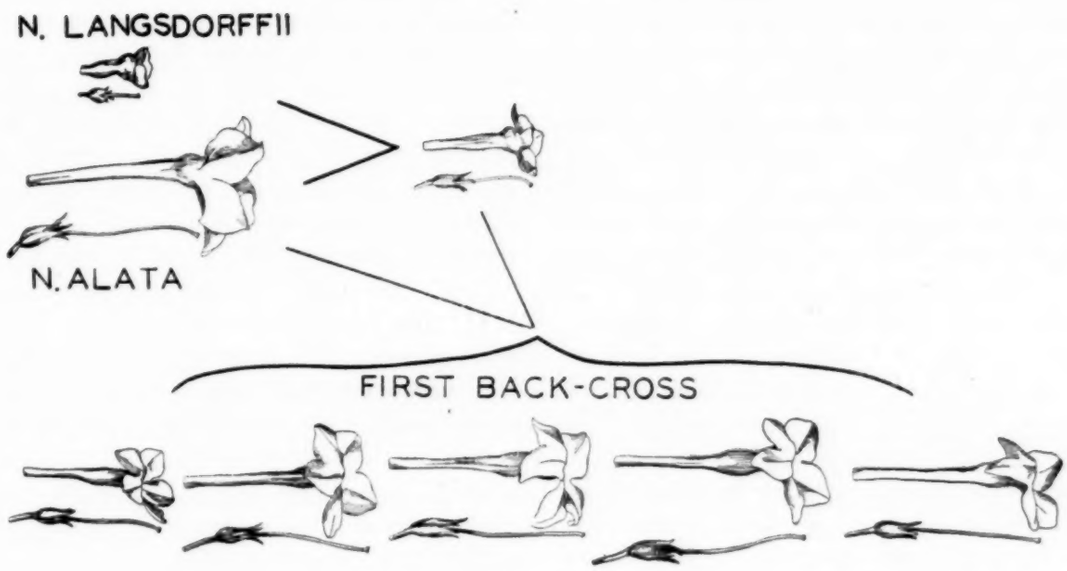

FIG. 1

name *introgressive hybridization* (Anderson and Hubricht, 1938), since it provided a mean by which elements in the germplasm of one species might introgress into the germplasm of the other. The chief result of hybridization under such conditions is the enrichment of variation in the participating species. Such hybridization is cryptic and only by very specialized techniques can we measure its exact importance in any particular case. Since the first publications on the subject, the phenomenon of introgressive hybridization has been confirmed, with experimental verification, in numerous genera. (A comprehensive bibliography on introgression by Dr. Charles Heiser is now under way. The following papers are representative: Goodwin, 1937b; Riley, 1938, 1939a, 1939b; Dansereau, 1941; Marsden, Jones and Turrill, 1946; Epling, 1947; Stebbins, Matzke, and Epling, 1947; and Heiser, in press.) Circumstantial evidence indicates its importance in many more genera. How important is it on the whole in the higher plants and in other groups of organisms? We do not yet have any exact evidence. From those cases where experimenters have gone to the trouble of making experimental backcrosses (Epling, 1947), it is clear that many of the second backcrosses could not be recognized as of mongrel origin purely by their appearance. It is therefore clearly indicated that gene flow from one species to another may go far beyond any point which could be detected by ordinary morphological techniques. We shall not be able to assess the real importance of introgression until we can study genetically analyzed species in the field and determine the actual spread of certain marker genes. Until such data are available, any generalizations are based on mere opinions.

Introgression therefore gives us a partial answer to our original question as to why hybrids are so seldom met with in nature. It is because when hybrids do occur, they usually perpetuate themselves, if at all, in backcrosses to one or the other parental species and the mongrel nature of their descendants is not apparent to the ordinary biologist. The commonest result of hybridization is introgression, and introgression must be excessive before it will produce results conspicuous enough to impress biologists who are not making a deliberate search for such phenomena. This is only half an answer. Why do interfertile species limit themselves very largely to backcrosses when they meet under natural conditions and most particularly why is the backcrossing

largely in areas where natural conditions have been very much disturbed? There are at least two main reasons; one resides in the germ plasm itself, the other in the habitat. As to the internal one, the total effect of all the forces which make for specific cohesion is very great, much greater than one would expect until he made careful calculations (Anderson, 1939a & b). Following the arguments used in these calculations it can furthermore be shown that in well-differentiated species the total effect of linkage is so strong that two well-differentiated but interfertile species, meeting in an idealized environment favorable to hybridization, would remain recognizable units in spite of their interfertility. The details of the argument are largely mathematical and are shortly to appear elsewhere, but the general conclusions can be tersely put. Linkage by itself is a force strong enough to prevent the complete swamping of interfertile species. As a factor in specific cohesion it is proportional to the differentiation of the two hybridizing entities: the greater the differentiation, the stronger the cohesive force of linkage.

The effect of the habitat, however, is also important, and it usually operates in exactly the same direction. The argument is as follows: it is now known that the physiological differences between species segregate in the same way as do the morphological ones. In Neurospora (Beadle, 1945) the mode of inheritance of scores of physiological differences are precisely understood. In yeasts the Lindegrens (1947) have demonstrated with laboratory precision the inheritance of various differences in habitat preference. The higher plants are not so amenable to precise physiological analyses of their nutritional requirements but there is abundant circumstantial evidence that a similar situation prevails and there are precise data for a few characters such as maturity (see for instance Goodwin 1937c, footnote 13, or Marsden Jones and Turrill, 1946), response to day length, etc. Nearly everyone who has grown and studied the second generation from a species cross has noted the segregation and recombination of such physiological differences as length of blooming season, resistance to diseases, dayblooming habit vs. night blooming, ease of wilting, resistance to cold, light tolerance, etc. If, therefore, we cross two species differing in their ecological requirements we may expect these physiological differences to segregate as follows: The first hybrid generation will be uniform in its requirements and on the whole they will be for conditions intermediate between those required for the two parents. The second generation will be made up of individuals each of which will require its own peculiar habitat.

Let us repeat this last statement; it is the crux of the argument. THE SECOND GENERATION WILL BE MADE UP OF INDIVIDUALS EACH OF WHICH WILL REQUIRE ITS OWN PECULIAR HABITAT FOR OPTIMUM DEVELOPMENT. As a whole the requirements of the second generation will range from a need for something more or less like one parental habitat to something more or less like the intermediate habitat of the F-1 to something more or less like the habitat of the other parent.

In nature therefore we might reasonably expect to find first generation hybrids growing in an intermediate zone between the two parental habitats. The persistence of any considerable variety of the various second generation recombinations would require a habitat such as is seldom or never met with, where various combinations of the two parental habitats are found in close juxtaposition to one another.

As a crude example let us consider the adjacent habitats in which one finds *Tradescantia subaspera* and *Tradescantia canaliculata* at home in the Ozark Plateau (Anderson and Hubricht, 1938). The former grows in deep rich woods at the foot of bluffs while the latter grows up above in full sun at the edge of the cliffs. As an over-simplified example we can

list three of the outstanding differences between these two habitats as follows:

rich loam.....rocky soil
deep shade.....full sun
leaf mould cover.....no leaf mould cover

Tradescantia canaliculata and *T. subaspera* are well-differentiated species; neither one of them is by any means the closest relative of the other, yet Mr. Hubricht and I have found by actual experiment that not only can they be crossed readily by artificial means but they do cross abundantly when left to themselves in an experimental garden (Hubricht and Anderson, 1941). Even though both he and I were familiar with the appearance of these artificial hybrids and though we searched for them at many points where the species were growing very near one another, we found very few of the first generation hybrids. The habitats of the two species are strikingly different; in the Ozarks one seldom finds the intermediate habitat in which the hybrid is able to germinate and survive. This is a more or less intermediate condition, a gravelly soil, partial shade with some bright sunlight, and a light covering of leaf mould. Imagine, however, the habitat which must be provided if we are to see the second generation recombinations which we obtain in the breeding plot. If we consider only the three contrasting characters of the habitat which have been mentioned above, our recombinations would require the following six new habitats in addition to the parental ones (these six represent only the extreme recombinations; a whole series of intermediates will also be required):

rich loam	rocky soil
full sun	deep shade
no leaf mould	leaf mould
rich loam	rocky soil
full sun	full sun
leaf mould	leaf mould
rich loam	rocky soil
deep shade	deep shade
no leaf mould	no leaf mould

What would have to happen to any natural area before such a set of variedly intermediate habitats could be provided? It has been very generally recognized that if hybrids are to survive we must have intermediate habitats for them. It has not been emphasized, however, that if anything beyond the first hybrid generation is to pull through, we must have habitats that are not only intermediate but which present recombinations of the contrasting differences of the original habitats. If the two species differ in their response to light, soil, and moisture (and what related species do not?) we must have varied recombinations of light, soil, and moisture to grow their hybrid descendants. Only by a hybridization of the habitat can the hybrid recombinations be preserved in nature.

The actual inherent differences in ecological preference will of course be much more diverse than in the crude example given above. The number of different kinds of habitats required by the hybrids will rise *exponentially* with the number of basic differences between the species. With ten such differences, around a thousand different kinds of habitat would be needed to permit the various recombinations to find a niche somewhere as well suited to them as the original adjacent habitats were to the two parental species. With only twenty such basic differences (and this seems like a conservative figure) over a million different recombined habitats would be needed. Under natural conditions anything like such a situation is close to impossible. Ordinarily it is only through the intervention of man that it is even remotely approached. Even in these cases the new "Recombination Habitats" will largely be limited to habitats pretty much like those required by one of the parental species, but which in a few characteristics approach the requirements of the other parent. We may expect that even in such disturbed habitats there will be back-up recombinations not greatly different from one of the parents which will most readily find an ecological niche suited to them.

One of the best demonstrations yet published of the way in which man can provide strange new niches of hybrid recombinations is that given by H. P. Riley (1938, 1939a) in his analysis of the hybridization between *Iris fulva* and *Iris giganticaerulea*, two species which differ strikingly in their color, morphology, and ecological adaptations (Viosca, 1935). In one of the localities which he studied in detail on the Mississippi delta, a series of long, narrow farms run straight back from the highway side by side, in the fashion set by the French settlers, with almost the precision of experimental plots. The original environment at that point was fairly uniform, but each man has treated his farm a little differently. It was strikingly apparent from Riley's study that the numbers and kinds of hybrids varied from farm to farm. Some had few or none, while others, even when adjacent, had hybrids in great quantities; there were significantly more of them where the meadows had been pastured. In one farm in particular, the little depression which ran parallel to the highway had been subjected to a series of operations. The trees and shrubs had not been entirely removed from this area, but it had been repeatedly cut over and had in addition been heavily pastured. It had a swarm of different hybrid derivatives, almost like an experimental garden, and the hybrid area went right up to the fenceline at the border of the farm and stopped there.

Nor is this an isolated instance. Viosca (1935) and other students of Louisiana irises have worked out in considerable detail the relation between the production of hybrid swarms of these conspicuously different irises and the churning and rechurning of the habitat by ditching, pasturing, lumbering, road-building, etc. It is only where man has hybridized the natural environments of the Mississippi delta that nature can find an appropriate lodging place for the hybrids she has created.

This dependence of interspecific hybridization upon the intervention of man has been described by a number of authors (Darrow and Camp,[1] 1945; Anderson and Hubricht, 1938). It was discussed in some detail by Wiegand (1935) in his paper on "A naturalist's experience with hybrids in the wild." Marie-Victorin has given a vivid description (1922, p. 32; 1935, p. 65) of its operation when the original flora of the St. Lawrence valley was largely replaced by fields and pastures. Epling, Stebbins, Dansereau, and their students have commented upon the connection in a number of different genera. Does this mean that introgression as a phenomenon is limited to the areas disturbed by man and that its results are mere artifacts and not genuine natural phenomena? I think not. Though freely admitting that nearly all the introgression which has been studied experimentally (for one exception see Dansereau, 1941) is of the nature of an artifact, I believe that at particular times, and in particular places, introgression may have been a general evolutionary factor of real importance.

Under the conditions of an experimental garden, natural selection among the progeny of a cross between species is much less severe than it is in nature. Though the optimum environments for the sister hybrids may be quite various, it is possible to raise the majority of them in one plot, providing that they are widely spaced and competition with aggressive weeds is kept at a minimum. The prevalence of iris hybrids on one or two of the farms described by Riley (1938) may have been due in part to the reduction of competition with other plants, particularly grasses, as well as to the variations in shade and moisture brought about by repeated recuttings and overpasturing.

There must have been various times, even without the intervention of man,

[1] Darrow and Camp also considered the reaction of hybridizing polyploid complexes with the environment. Polyploidy introduces further complications into hybridization which are beyond the scope of this paper.

when species hybridized under conditions which produced varied new habitats and when competition was not too keen, as for instance when newly colonizable areas emerged from the sea or when various floras spread out onto the northern lands denuded by Pleistocene glaciation. At such times introgression would have been an important evolutionary factor. For one area we are beginning to get actual proof that it did occur. Along the coast of California there are peninsulas which once were isolated islands but which are now united with the main land. In their studies of the California knobcone pines, fossil and living, Mason and his students are demonstrating the actual role of introgression (for a general summary see Cain, 1944, pp. 112–118) in forming these pines as we know them today and to determine in some detail how introgression operated at the time when these islands were joined to the coast.

The Edwards Plateau in central Texas is another area in which introgression may have operated on a grand scale. This comparatively small area is a center of distribution and variation for numerous genera. A mere leafing through of a series of monographs of North American genera (Larsen, 1933; Anderson and Woodson, 1935; Barkley, 1937) will demonstrate that it is one of the outstanding centers east of the Rockies. For many genera the concentration of species is higher there than at any other point and for the genus Tradescantia we know the even more significant fact that it is a center for the diploid strains of polyploid species (Anderson and Sax, 1936; Anderson, 1937). The geological evidence shows that when the Edwards Plateau came into being, it united older land masses in Mexico and in the United States. Certainly at such a time related species in many genera might have met and hybridized under conditions where competition would not have been keen and where associations of plants were in the making instead of already existing as tightly closed corporations. Tradescantias from Mexico would have met species coming down from the Appalachians in an area conducive to the survival of some of the hybrid recombinations.

Woodson has recently (1947) called attention to the importance of peninsular Florida in the speciation patterns of the eastern United States. During parts of the Tertiary it was an island or group of islands which finally became attached to the mainland. Species and varieties which became differentiated during the island period must then have had unusual opportunities to hybridize with their relatives on the mainland. Giles' (1942) studies of Cuthbertia in this area have given cytological proof that such hybridization did actually take place. Careful studies of variation throughout the whole area, for a series of species, should yield data with which we could assess the general overall importance there of introgression.

Summary

1. Experimental evidence shows that sterility will not account for the rarity of hybrids under natural conditions.

2. Careful field analyses have shown that natural hybridization is largely limited to backcrosses which resemble the parental species so closely that special methods are required to detect them readily.

3. One of the factors limiting hybridization to such introgression is imposed by the habitat for the following reason: Two species differing in their habitat requirements will produce a first generation hybrid adjusted to a uniform intermediate environment. The second generation however consists of individuals each of which requires its own peculiar habitat for optimum development. Such heterogeneous habitats are seldom or never met with, the only approach to them being found in places where man has greatly altered natural conditions.

4. It is concluded that hybrid swarms can survive only in "hybridized habitats." While most of the latter result from hu-

man intervention, similar conditions have prevailed in pre-human times when new lands were opened up to colonization by diverse floras. At such times and places introgressive hybridization must have played an important role in evolution.

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NATURAL HYBRIDS OF SUBSPECIES OF HIPPODAMIA QUINQUESIGNATA (COCCINELLIDAE) [1]

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Received November 22, 1947

So much of the study of evolution is now being done in the laboratory that it behooves all who observe comparable phenomena in nature to put them on record. Whether the phenomena observed out of doors are actually examples of an evolutionary process must usually be decided by inference, at least in large part, and the one here recorded is no exception to this rule.

The Original Observation

What was at first hoped would prove to be a species cross was observed in a newly collected hibernating mass of lady beetles sent by Professor H. B. Yocom. They were collected on Spencer Butte, five miles south of Eugene, Oregon, at an elevation of 2200 feet, on April 6, 1942. Such masses tend to be composed of a single species. Of over 23,000 beetles in this mass, nearly all were *Hippodamia sinuata spuria*, whose pattern of heavy black spots (fig. 2, *C*) rests on a background of ochraceous buff (Ridgway). This species has a spotless phase, with which the typical spotted beetles cross freely (Shull, 1943). Among these black-and-buff beetles were somewhat over a hundred having a red ground color, mostly with few spots or none (fig. 2, *A*). Nearly all of these, as identified by Dr. E. A. Chapin, to whom I wish to express my thanks, were *Hippodamia quinquesignata ambigua*. The form *ambigua* was formerly regarded as a distinct species, but is listed as a subspecies of *quinquesignata* in Chapin's recent review (Chapin, 1946).

In the hibernating mass a spotless female *ambigua* was found copulating with a male *sinuata spuria*. The pair was removed, and continued copulating for more than an hour after its isolation. It was recognized that the female might have mated earlier with one of her own kind, even before entering the hibernating mass in the fall; but when some of the descendants of the female were distinctly spotted, it was first believed that the male *sinuata spuria* had shared in producing them.

Two circumstances, however, made it necessary to reject the *spuria* male as the source of spotting. First, none of the subsequent generations appeared to show any influence of the ochraceous buff ground color. All the descendants of the female in question had red ground color; and while there were variations in this color, none of them suggested a buff contribution. Exclusively red descendants could, it is true, be explained by assuming that ground color is in some sense a strictly maternal character. An attempt was made to test this possibility by mating female *spuria* with male *ambigua*, but all such attempts failed. This failure does not prove too much, since attempts to cross species in the lady beetles seldom succeed; and besides, a number of attempts to repeat the cross in the same direction (*ambigua* female × *spuria* male) likewise failed. When these attempts were made the beetles had already been inbred several generations, and everyone who raises the coccinellids seems to find the same rapid decline of vigor with in-

[1] Contributions from the Department of Zoology, University of Michigan. Aided by a grant from the Faculty Research Fund and a gift from Mrs. S. Ralph Lazrus.

breeding (Shull, 1944). Consequently, the failure to effect the species cross was then regarded as possibly no more than a failure of any cross, even within one of the species.

A more conclusive reason for rejecting any *spuria* contribution was the fact that all male descendants of the *ambigua* female had *ambigua* male genitalia. No one will know what kind of genitalia a hybrid of these species should have until the cross is made; but there should have been some kind of segregation in later generations, and there was none. A cross between *Hippodamia convergens* and *H. quinquesignata* (Shull, 1946a) resulted in intermediate genitalia in the hybrids, and subsequent segregation suggesting perhaps a three-gene differential between them. The absence of any sign of the *sinuata spuria* type of genitalia in the descendant males made it definitely necessary to conclude that the observed interspecific mating had not produced hybrid offspring.

What, then, was the source of the spotting of some of the descendants of the spotless *ambigua* female? The facts from which the answer to this question must be drawn by inference are presented in the following pages.

The Pedigree of Spotting

From her own progeny the copulating *ambigua* female must be regarded as herself heterozygous for spotted, and it must be concluded that she had mated with a similarly heterozygous spotless male. The reasons for these assumptions are more precisely stated below. She is listed, therefore, as one member of a pair of spotless × spotless. Two other matings of the red beetles, guests in the hibernating mass of *sinuta spuria,* were made, both of them spotless × spotless. It is assumed, though not proven, that the males used were the fathers of the offspring obtained. These three females gave rise to the lines of descent, partly intermingled, shown in Figure 1. The female found copulating in the hibernating mass gave rise to the progeny numbered 155; the male parent is unknown but is inferred to have been spotless, heterozygous for spotted. In the other two pairs, leading to progenies 157 and 151, respectively, the male is known unless the females had mated before isolation.

In this chart, below the experiment number, are given the numbers of spotless and spotted individuals, the spotless (dominant) being shown first. Above the experiment number, the light sloping lines lead to the source or sources of the parents. It seems unnecessary, in this study, to distinguish the sexes of the parents, but whether they were spotted or spotless is indicated by the groups to which the light lines lead. Beside these lines are the genotypes assigned, sometimes with doubt, to the parents. *S* is the gene for spotless, *s* for spotted. In Experiment 166, which was first intended merely as a stock, two males were used, and two genotypes are given on the chance that one of the males might have been homozygous. If the female had mated with both a homozygote and a heterozygote, the small number of spotted offspring (distinctly less than one-fourth) might be explained.

Criterion of Spotted and Spotless

As is well known, "spotless" Hippodamia may, and often does, have spots. The only criterion yet established for distinguishing the amount of spotting proper to the spotted pattern from that possible in "spotless" beetles is the one determined (Shull, 1944, 1946b) in experiments with Michigan beetles of the species *Hippodamia convergens.* There is no direct evidence that the same standard can be applied even to other populations of *H. convergens,* since the validity of it would be affected by the number of modifying genes (judged to be three or four pairs in the Michigan population). Certainly there could be no assurance that this standard would apply to other species. Nevertheless, when this criterion was applied to the experiments with the

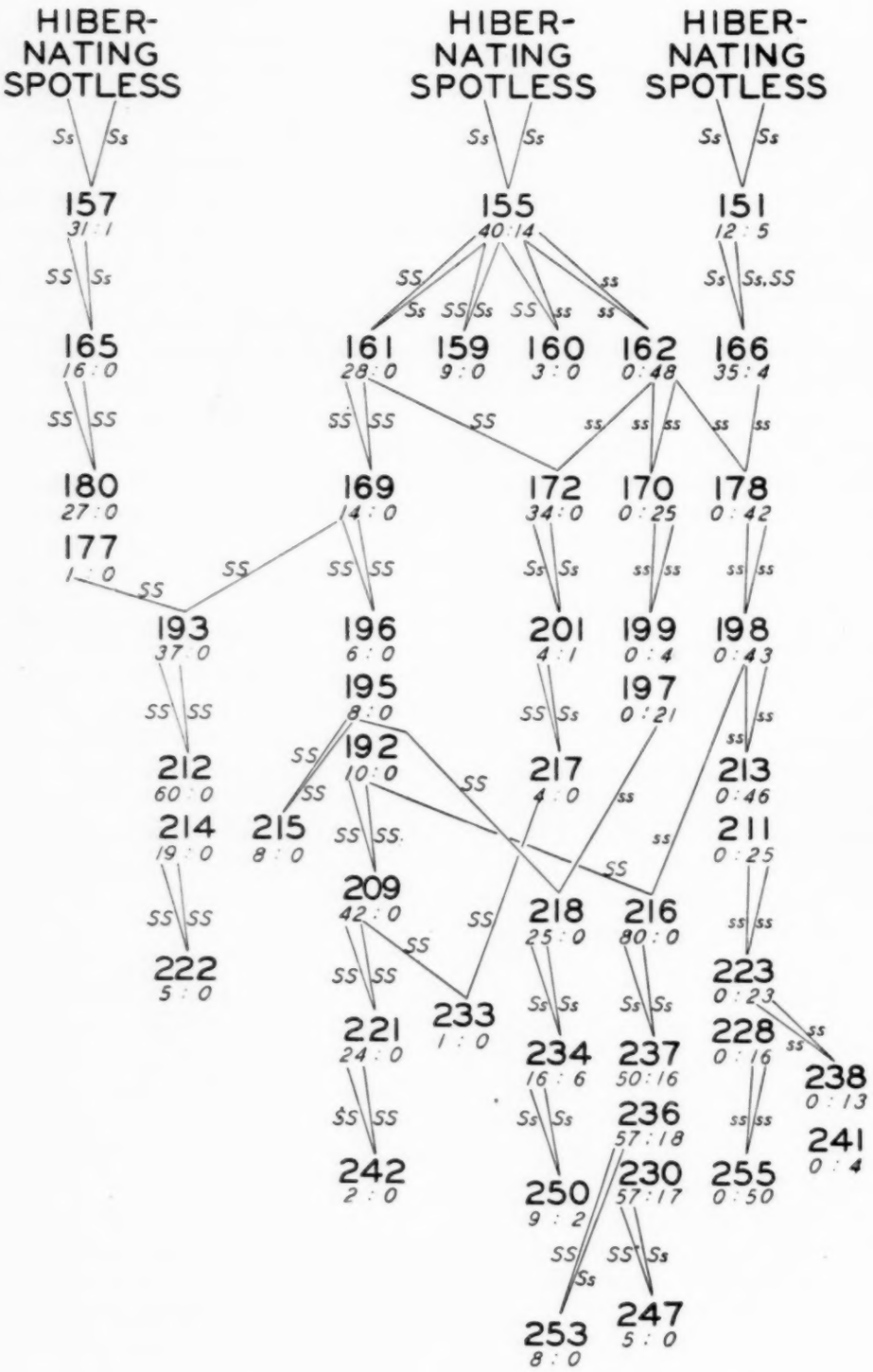

FIG. 1. Course of the experiments. Under the experiment numbers are the ratios of spotless to spotted beetles, spotless being given first. Over the experiment numbers the sloping lines indicate the source of the parents with the genotypes of the parents beside these lines.

Oregon beetles of this paper, in matings which were known to be between two heterozygous spotless individuals, reasonably close approximations of the 3:1 ratio were obtained. In the absence of any other pertinent data, therefore, the Michigan standard has been used.

Any such standard is strictly valid only in a statistical sense, which is sufficient where ratios of spotless and spotted individuals are wanted. Almost certainly it would result in some mistakes concerning individuals. The dividing line between spotless and spotted was a total size rating of the three posterior spots of 7.75 in a possible maximum of 12. Beetles having posterior spots larger than 7.75 were called spotted, those with smaller posterior spots were considered spotless.

Some information concerning the amount of overlapping between the amount of spotting of spotless and spotted beetles of the Oregon population can be gleaned from the experiments here reported. On the right side of the chart in Figure 1 are several lines of descent whose individuals must be "spotted" regardless of the size of their posterior spots. The details of these patterns cannot be given here for the hundreds of beetles because of space limitations, but some of the least spotted may be recorded. In progeny 162, which must be considered wholly spotted, the six spots of one beetle were rated 003130, another 001121 (see Shull, 1944, for the scheme of rating). The three posterior spots had a combined rating of only 4, in a possible maximum which for this strain was 13 instead of 12. In Experiment 170, descended from 162, the least spotted beetle was 303330. In 197 and 199, descended from 170, the least spotted individual was 412133. In progeny 178, likewise all spotted, the three least spotted were 212120, 100220, and 133130. In 198, inbred from 178, the least spotted was 301332. In the seven experiments descended from 198, only three beetles were as lightly spotted as the minimum one in 198. From their paucity one might suspect their reduction of spots was caused by developmental accidents, not genes.

In each of these two lines of descent the minimum rating shows a tendency to increase, as if modifying genes responsible for the reduction were being accidentally bred out.

Among the spotless lines of descent, the one descended wholly from 161 included only four beetles as much spotted as 000200. In progenies 177 and 180 the most spotted beetle (so far as the posterior spots were concerned) was rated 000100; but 165, from which they were bred, included one rated 030312.

These statements regarding the criterion of spotting are of importance chiefly in relation to one individual in progeny 157. The rating of spots in this beetle is 040433, and it was regarded as spotted. Its three posterior spots have a combined size rating of 10, which is above the adopted dividing line. It is the only member of 157 which meets the requirement of the genetically spotted pattern; the ratio of spotless to spotted is accordingly recorded as 31:1, and the parents are assumed to be both *Ss*. The ratio is, however, so far from 3:1 as to raise the question whether this beetle were not genotypically spotless. Of the known spotless beetles of these experiments, none has posterior spots as large as 10; later in this same line of descent, as indicated above, was one with posterior spots totaling 6. Nowhere else in all the experiments was any beetle, known to be spotless, more heavily spotted than that.

Balancing the poor approach to the 3:1 ratio in 157 against the wide discrepancy between the posterior spotting of one beetle and the criterion of spotlessness, one must presumably leave in doubt whether the beetle recorded as spotted in 157 really is such.

Number of Heterozygous Guest Beetles

The principal significance of the discussion just closed is its bearing on the frequency of heterozygosis among the spotless red beetles hibernating with *H. sinuata spuria*. Six of these beetles were bred. Certainly four of them, the parents of 151 and 155, were heterozygous. The parents of 157 were both heterozygous if one of their offspring was spotted. If all the offspring in 157 were spotless, either or both of the parents were presumably homozygous. It is rather unlikely that both were homozygous; for if the six beetles chosen for breeding consisted of four heterozygous and two homozygous, there is only a minor chance that the four heterozygotes would have been arranged in two pairs and the two homozygotes in one pair. From these considerations it is concluded that at least four, probably as many as five, and possibly all six of the spotless beetles which were bred were heterozygous.

From this conclusion it seems probable that a very considerable majority of the guest beetles, perhaps all of them, were heterozygotes. The possible significance of this frequency of heterozygotes is indicated later.

Postmedian Band

One feature of the spotted pattern needs to be separately treated. This is the fusion, in some of the beetles, of spots 4 and 5 into an oblique postmedian band (fig. 2). This band had already been studied in crosses between *Hippodamia quinquesignata* and *H. convergens* (Shull, 1945). In those hybrids the crosses had not borne too closely upon the mode of inheritance of the band, but on the whole the results were in best conformity with the conclusion that fusion of the spots into a band is recessive to their separation as distinct spots.

That conclusion is more definitely supported by the results reported here. In progeny 155, of the 14 spotted individuals (see fig. 1), 9 had spots 4 and 5 separate, 5 had them joined. The parents of 162 were chosen from among the 9 whose spots were separate; their offspring included (among the total of 48 spotted) seven with spots 4 and 5 definitely joined and an additional one in which these spots were loosely or doubtfully joined. From among the other 40 with separate spots were taken the parents of 170, among whose 25 spotted offspring only two had the spots joined, and these only doubtfully. Separate-spotted offspring of 170 gave rise to progenies 197 and 199, whose 25 spotted offspring (plus 5 not appearing in the chart because, through a confusion of records, they could have belonged to either 197 or 199) consisted of 22 spotted and 8 (one doubtfully) banded.

The original pair producing progeny 151 also possessed the banding factor, for, though none of the five spotted members of that progeny was banded, one of

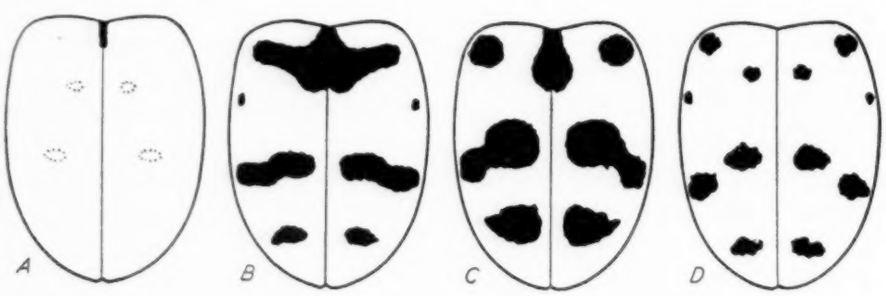

Fig. 2. Patterns of the elytra of four subspecies or species of Hippodamia. *A, H. quinquesignata ambigua; B, H. q. quinquesignata; C, H. sinuata spuria; D, H. convergens.* The dotted rings in *A* represent occasional spots which might occupy any of the six positions of spots in *D*.

the four in 166 (derived from 151) had spots 4 and 5 joined. Progeny 178, whose parents (one from 162, the other from 166) both had separate spots, included 23 separate-spotted, 11 banded, and 8 with the spots loosely or doubtfully joined. This ratio would probably better fit the assumption that one of the parents of 178 was homozygous for banding, but neither of them showed any sign of fusion.

Beyond this point in the pedigree is the first mating of two banded parents. The parents of 198 both had spots 4 and 5 distinctly joined. Of all this progeny and the seven others descended from it, each of them from two banded parents, every beetle except three was banded, and these three had spots 4 and 5 loosely joined.

From all the above data it seems necessary to attribute fusion of spots into a postmedian band to a recessive gene. In addition, however, there must be other genes modifying its expression to some extent. Both parts of this conclusion are in agreement with the earlier work on species hybrids (Shull, 1945).

Source of Spotting

In his review of the genus Hippodamia in North America, Chapin (1946) lists thirteen or fourteen subspecies, belonging to eleven species, having a distribution which would make them at least conceivable contributors to natural hybrids near Eugene, Oregon. It seems unnecessary to look outside the genus, since even species crosses, so far as now known, are infrequent. Among the subspecies available in that area, the probable source of the spotting in the hibernating guests reported here can be inferred only from such things as their relative abundance and the genetics of the pattern of the elytra and prothorax and of the shape of the male genitalia.

If the abundance of individuals is reflected in the number of collections which were available for Chapin's study, the ones that stand out as probable contributors to a cross are *Hippodamia quinquesignata quinquesignata, H. q. ambigua, H. convergens,* and *H. sinuata spuria.* The guests in the mixed hibernating mass were judged to be of the second of these subspecies, their hosts were of the last-named kind.

The convergent white marks on the prothorax, which all the guest hibernators and all their descendants had, could have come from all four of the abundant subspecies named above, and from three others —*H. moesta moesta, H. caseyi,* and *H. sinuata sinuata.* Nothing is known of the mode of inheritance of this pronotal pattern, but whether it is simple or complex there would presumably be some indication of segregation in the later hybrid generations unless the original pair of parents were alike with respect to it. Accordingly it is assumed that the unknown parents of the guest hibernators must both have had the convergent white marks.

The six spots on each elytron could probably be furnished by most of the species which are geographically so placed as to be considered. So many of the Hippodamia species which characteristically have bands occasionally have these bands broken up into spots that it seems likely that the spots form a common, genetically basic pattern (fig. 2). Of the geographically accessible types, according to Chapin's studies, the ones showing most definitely the six full spots on each elytron are *H. tredecimpunctata tibialis* and *H. convergens,* though *H. quinquesignata quinquesignata* sometimes has separate spots rather than the anterior and postmedian bands.

The fusion of spots 4 and 5 into a postmedian band, shown in the entire line descended from Exp. 178 and in portions of other progenies, must have been represented in the original parents. The guest hibernators were heterozygous for it, just as they were heterozygous for the general spotting. Marked fusion of these spots, without other bands, occurs in *H. sinuata spuria,* and sometimes in *H. q. quinquesignata* and *H. caseyi,* though the

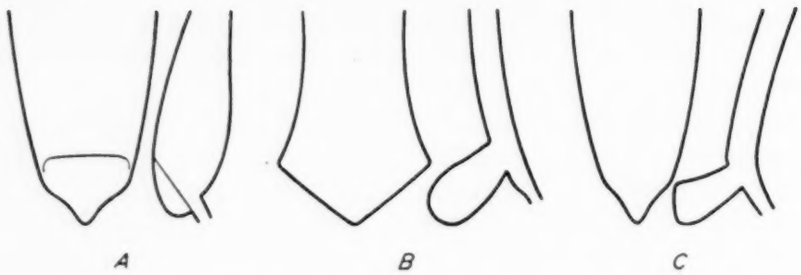

FIG. 3. Male genitalia of Hippodamia species, aedeagus at left in each species, distal end of sipho with flaps at right. *A, H. quinquesignata; B, H. sinuata; C, H. convergens.* Subspecies do not differ from the typical forms in these structures.

shape of this band in the hybrids here reported was similar only to that of *quinquesignata*. The other forms available in Oregon do not ordinarily have a band of this shape, nor this band without other bands.

The male genitalia of the hybrids appear to exclude all of the accessible species except *H. quinquesignata* as the source of spotting. The inheritance of coccinellid genitalia is known only for the cross of *H. quinquesignata* with *H. convergens* (Shull, 1946a), as stated earlier. In this cross, the specific difference in the male apparatus could rest on as few as three pairs of genes, without dominance and cumulative in their effect as between pairs. This distinction would account for the intermediate nature of the first-generation hybrids and the rapid approach toward the two parent types on repeated backcrossing. Intermediacy of the F_1 of *H. parenthesis* and *H. lunatomaculata* was reported by Timberlake (1919), but no further generations were reared.

On the basis of these known facts one would expect the hybrids reported here to show a similar intermediacy in F_1 with segregation in later generations, but there was none. The male genitalia of all the hybrids, in all generations, were like those of *H. quinquesignata*—that is, like the males of the species to which the known female progenitors of these hybrid lines were assigned. All the genitalia have the sloping transverse keel or carina across the aedeagus; all have the heavy sipho, and low siphonic flaps shaped in outline like a low skew probability curve (fig. 3, *A*). The genitalia of no other species are nearly enough like these to constitute a likely source. Both of the hybridizing types must have carried genes for this form of genitalia.

From the evidence presented in this section it seems necessary to conclude that the guest hibernators were hybrid offspring of a spotted and a spotless *H. quinquesignata,* presumably *H. q. quinquesignata* and *H. q. ambigua.* One of these parents must have carried the gene for the fusion of spots 4 and 5 into a postmedian band. If either of them carried the gene for fusion of spots 1 and 2 into an anterior band, it could hardly have been homozygous for such gene, since none of the descendants showed any indications of this band.

SOURCE OF THE BANDING

Most specialists would doubtless conclude that the gene for fusion of spots 4 and 5 entered with *H. q. quinquesignata,* which typically has such a band. One cannot rule out, however, the possibility that *ambigua* also has such a gene. Were a *quinquesignata* to acquire the spotless gene, which several species of Hippodamia possess in some individuals, and which can be transferred from *H. convergens* to *H. quinquesignata* without any apparent modification of its inhibiting effect on pattern (Shull, 1945), it is likely that such an individual would be identified as *ambigua.* What genes for spot-

ting the natural populations of *ambigua* possess cannot be told, of course, until genetic work, involving a fortunate mutation in that subspecies or a known cross with one of the spotted subspecies, has been done. Geographic separation of a *quinquesignata* population homozygous for the spotless gene could easily lead to the recognition of a spotless subspecies *ambigua*.

Why H. Quinquesignata Guests of H. Sinuata?

An important question, not yet answerable, is why the hundred or more individuals of *H. quinquesignata ambigua* were guests of the thousands of *H. sinuata spuria*. The mere fact of aggregation in winter, when hundreds of places for hibernation are available to single specimens, indicates presumably some attractive quality and a sense that responds to it. The additional fact that such masses are made up, in general, of a single species suggests that the quality and the capacity to sense it are specific in their nature.

When a main mass of one species is accompanied by a few individuals of another species, is that just an accidental result? Especially, is it accidental when the guests are found to be hybrids?

The idea that the hybrids discussed in this paper might have inherited a dominant sense from the species to which they were attracted in hibernation was one reason for supposing that one of their parents was *H. sinuata spuria*. As clearly shown, however, neither the pattern, nor the ground color, nor the genitalia of the descendants permit the assumption that the hybrid guests were offspring of a cross involving *H. sinuata*.

An alternative possibility is that certain species of Hippodamia, but not all of them, possess the mechanism of attraction, perhaps in different degrees. If *H. q. quinquesignata* should be sometimes found hibernating with *sinuata*, while pure *H. q. ambigua* never is, this distinction might be traceable to an attraction pair partly common to *quinquesignata* and *sinuata* but not present in *ambigua* at all. Such a distinction would account for guest hibernation of the subspecific hybrids here described; it would also help to account for the observed isolation of *ambigua* from typical *quinquesignata*, which mere possession of the spotless gene could hardly explain.

Summary

Some spotless red beetles assigned to the subspecies *Hippodamia quinquesignata ambigua*, and found hibernating with *H. sinuata spuria*, were shown by breeding experiments to be heterozygous for a spotted pattern. The spotting could not have come from *spuria*, their host, because the genitalia of the males among the hybrids were exclusively of the *quinquesignata* type, and because there was no recovery of the buff ground color of *spuria* in any of the descendants of the *ambigua* hybrids.

Most if not all of the guest hibernators must have been thus heterozygous, suggesting that their heterozygosis might be the reason for hibernating with *sinuata spuria*. Some dominant feature (perhaps an odor) of the mechanism which draws members of one species together in hibernation could be responsible for similar attraction in hybrids. However, since *sinuata* could not be one parent of the hybrids, other species must possess a similar attractive quality if this be the explanation of guest hibernation. Such a quality or the lack of it would be a more plausible reason for isolation of *ambigua* from *quinquesignata* than would the spotless gene which is their visible distinction.

It is concluded, partly from the pattern, that *H. quinquesignata quinquesignata* and *H. q. ambigua* were probably the parents of the hybrids. The experiments furnish confirmation of an earlier doubtful conclusion that the fusion of two spots into a postmedian band is caused by a single recessive gene. The hybrids must have been heterozygous for this gene. *H. q. quinquesignata* typically has

the fusion gene; hence *ambigua* most probably lacks it.

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SOME ASPECTS OF EVOLUTIONARY THEORY IN THE LIGHT OF RECENT WORK ON ANIMAL BREEDING

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Received November 10, 1947

The most significant recent development in studies of selective breeding in domestic animals lies in the extension and application of the principles of population genetics to the problems of artificial selection. Occasionally it happens that an applied field repays its debt to fundamental or pure science by suggesting further lines of inquiry, by developing new techniques of study, or by supplying empirical evidence for testing both deductive and experimentally derived theories. It would be presumptuous to advance such broad claims for the situation in question. Nevertheless, it may be ventured that some of the results obtained in recent studies in animal breeding have considerable illustrative, if no other, value to students of evolution. The purpose of the following remarks is, therefore, to call certain techniques and conclusions which may have a bearing on evolutionary theory to the attention of those students who do not follow closely zootechnical literature.

Natural selection entails differential survival or reproduction, which is in turn determined by the properties of phenotypes, that is, by the morphological and physiological characteristics of individuals. The consequent differential reproduction of phenotypes leads to a differential reproduction of genotypes, in a manner and to an extent determined by the complex causation of phenotypic differences by both environmentally and genetically elicited differences. Genotypic changes in turn can produce gradual phenotypic changes in a population, the nature and degree of which will be determined in part by the breeding structure of the particular population concerned.

Some of the recent laboratory work bearing on evolution has been concerned mainly with the relations between *genotypic* differential reproductive rates, population structures, and the consequent rates of change or equilibrium conditions. As a prime example of this approach Dobzhansky's (1947) experiments on the selective values of alternative inverted chromosomal segments in *Drosophila* may be cited. An outstanding instance of a quantitative analysis of *phenotypic* differential reproductive rates in a natural population involving character alternatives due to a single gene difference is Gershenson's (1945) study on melanism in hamsters. Finally, the investigations of Clausen, Keck, and Hiesey (1940) may be recalled as an example of a careful study of the degree to which metric characters in plants can be modified by environmental conditions.

There is, however, a notable lack of studies on the environmental and genetical determination of continously variable characters in relation to the effect of selection on natural populations. There has also been little, if any, quantitative work on the results of simultaneous selection of several characters under natural conditions. It is in these particular fields with respect, of course, to domestic rather than natural populations, and artificial rather than natural selection, that students of animal breeding are forced to operate.

As it must be the case with metrical characters, the treatment pursued in the present discussion is a mathematical one, essentially based on the application of Wright's (1921) method of path coefficients. The demonstrations attempted may therefore be somewhat difficult to

follow for those not thoroughly conversant with this technique. Hence, a non-mathematical summary is appended for their benefit.

The Degree of Heritability

Two postulates, which have been found to be useful approximations in animal breeding problems, are that gene differences have strictly additive effects and that genetic and environmental influences interact in an additive manner. The first of the assumptions implies that any given allele produces a constant phenotypic effect irrespective of the rest of the genotype of the organism. The simplest of the non-additive deviations is dominance, while the other types are conveniently referred to *en masse* as epistatic deviations. The second postulate similarly assumes that the interactions between the genotype and the environment are linear in nature. With respect to metrical characters, whose expression is conditioned by many genes each of relatively small effect, these postulates seem to be warranted. In general, the effects in a population of even completely dominant gene differences, as well as differences exhibiting certain types of epistatic effects, can usually be almost completely accounted for in terms of additive effects (Wright, 1935a; Lush, 1945). In terms of these postulates the total variance may be partitioned by the methods devised by Wright (1921) into a genetic fraction, h^2, and an environmental fraction, e^2, in which case $h^2 + e^2 = 1$. A varying proportion of such non-additive effects as may exist will appear in the environmental fraction e^2, depending on the methods of partitioning used. The different methods of determining h^2 from actual data are illustrated by Whatley (1942), while Dickerson and Grimes (1947) discuss in some detail the extent and type of bias to which these various techniques are subject. In general, these methods are based either on the measurement of phenotypic resemblance between individuals of various degrees of genetic relationship, or on the observed changes

Table 1. *Explanation of symbols*

a	The relative importance of a given trait to the aggregate value of an individual (see I). The value of a is expressed as the change in I that would result from one unit change in the trait.
e^2	The proportion of the total variance of a trait not accounted for by h^2 (*q.v.*).
G_X	The genotype for the trait X. Thus G_K is the genotype for keel length, and G_S the genotype for shank length in the example illustrated in figure 1.
h^2	The degree of heritability, or the proportion of the total variance of a character which is accounted for by additively acting gene differences. The sum of h^2 and e^2 is equal to unity. Heritability can also be defined as the square of the correlation coefficient between genotype and phenotype.
H	The genotypic value of an aggregate of traits.
i	The selection differential, or the superiority of the individuals leaving offspring over the average of their own generation for any trait considered. The selection differential is weighted for the relative number of offspring each parent leaves.
$\bar{\imath}$	The selection differential expressed not in actual units in which the trait considered is measured, but in standard deviations.
I	The index value of an individual with respect to the trait or complex of traits considered. It represents the phenotype on which selection operates. In the case of natural selection the index is merely the sum of the phenotypes of its component traits, weighted for the respective contribution (a) that each trait makes to the value of the aggregate. In artificial selection the weighting is ideally such as to provide a maximum increase in H.
K	The phenotypic value for keel length in the example in figure 1.
r	The coefficient of correlation. Thus $r_{G_S G_K}$ is the coefficient of correlation between the genotypes for keel length and shank length in figure 1.
S	The phenotypic value for shank length in the example in figure 1.
X	The phenotype for any trait X.
ΔG	The rate of change per generation in the average value of a genotype of a population under selection.
ΔH	The rate of change per generation in the average genotypic value of aggregate of traits.
σ^2_X	The variance (square of the standard deviation) of trait X.
$\sigma^2_{G_X}$	The variance of the genotypic values of trait X.

in populations under selection of known phenotypic intensity. The genetic fraction of the total variance, h^2, is usually referred to as the degree of heritability, or simply as the heritability (a fuller definition of this and all following symbols appears in table 1).

Let us consider some simple metrical character subject to selection in a natural population. The average advantage in actual units of measurement which the individuals leaving offspring (the parents-to-be) have over the mean for this character of all individuals of their generation may be designated as i. This is the selection differential and represents a measure of the intensity of selection. In the simplest possible case of non-overlapping generations where there is no differential mortality prior to reproduction, and where equal numbers of offspring are produced by each of the selected parents, i simply equals the difference between the mean of the parental phenotypes and the mean of all phenotypes of their generation. However, in general, there is some difficulty in arriving at the precise definition of this advantage. It represents the difference between the mean value of the parents-to-be, weighted in proportion to their relative contribution to the number of offspring they leave (as well as adjusted for the relative length of the reproductive cycle) and the unweighted mean for their generation. The weighting factor will be recognized as Haldane's (1932, pp. 172–174) definition of fitness, a definition which we wish to emphasize at this point, since it enters into much of the subsequent discussion. If there is differential mortality prior to the age at which measurement of the character concerned can be made, further adjustment in the computation of the selection differential will be required. This matter will be discussed briefly further in the section on sequential selection.

If no selection occurs it may be expected that the mean of the succeeding generations will remain at a constant level. With a selection differential of i, however, the mean of the offspring will equal that of the parental generation plus that fraction of i which is due to the genetic component of variance, namely ih^2. In other words, the weighted mean genotypic advantage of the parents, and hence the increase in the mean value of the offspring over that of the previous generation will be ih^2. With respect to any character X, the component of $\sigma^2{}_X$ attributable to genetic differences is $h^2\sigma^2{}_X$, which may be designated as $\sigma^2{}_{G_X}$ or as the genetic variance of X. The gain ih^2 may be then rewritten as $\frac{i}{\sigma^2{}_X}\sigma^2{}_{G_X}$. These expressions indicate that for a given phenotypic variance and selection pressure, the rate of change in X is directly proportional to the heritability of X or to its genetic variance. It may be noted that the principles underlying this theory have been experimentally substantiated at least in one case (egg production in chickens, as reported by Lerner and Hazel, 1947).

Selection for the Aggregate Genotype

Ordinarily in nature selection operates not on single phenotypic characters but on the combination of all of the adaptive traits. It is possible to introduce an abstract notion of an aggregate genotypic value and to determine changes both in this aggregate and in the components contributing to it under the effects of selection. According to the theory developed by Hazel (1943) the rate of change in the aggregate genotypic value (which may be designated as H, and whose analogue in natural populations is fitness) is equal to the sum of the products of changes in each of the components of such an aggregate and their respective contributions to the aggregate. Thus if the values of the genotypes of traits 1, $2 \cdots n$ are respectively designated as G_1, $G_2 \cdots G_n$, and their respective contributions to H are a_1, $a_2 \cdots a_n$,

$$H = a_1G_1 + a_2G_2 \cdots a_nG_n,$$

and

$$\Delta H = a_1\Delta G_1 + a_2\Delta G_2 \cdots a_n\Delta G_n.$$

In turn the various ΔG's are a function of their heritabilities and the selection intensities for them. However, when the different components of the phenotypic aggregate are correlated with each other, each ΔG depends not only on the selection differential (i) and heritability (h^2) of the corresponding trait, but also on the magnitudes of the environmental and genetic components of the correlations between it and the other traits. A positive *genetic* component of correlation between two desirable traits usually tends to increase the rate of genetic improvement under natural selection, because selection for each trait by itself results in improvement in both traits. A positive *environmental* component of correlation between desirable traits, on the contrary, will hinder genetic progress. Conversely, a negative environmental component of correlation between two desirable traits or, what amounts to the same thing, a positive environmental component of correlation between a desirable and an undesirable trait facilitates progress. In the latter case, selection for each character constitutes a partial compensation for the effect of environmental differences on the other trait. Direct phenotypic selection, such as usually occurs in nature, acts on the phenotypic aggregate, which may be conceived as a selection index in which the component traits are weighted entirely according to their respective contributions to H, or, in other words, their respective values of a. On the other hand, under artificial selection an index can be constructed in which the weights assigned to individual components can be chosen by taking into account the degree of heritability of each trait and the correlations between the traits in such a fashion, as to maximize the rate of change of the aggregate in the desired direction (Hazel, 1943).

In artificial selection the relative economic values of the different traits, and, consequently, the a values, sometime change with the passage of time (see Lush, 1936, for outstanding examples of such changes in the case of swine in Denmark). In nature the relative contributions of different traits to fitness may vary likewise in response to environmental conditions. Under both types of selection changes in the relative emphasis given to the various traits permit the utilization of hitherto unexploited sources of genetic variability. The following discussion applies to a simple situation where such changes in a values as occur are gradual, and are between rather than within single generations. Where the a values are significantly altered within a single life cycle, a form of sequential analysis (see below) is necessary.

Efficiency of Selection Indexes

Nature thus cannot be expected to devise an optimum selection index, and hereditary fitness, no matter how strong the natural selection pressure, does not change at the highest rate possible. There will be, instead, over-emphasis in selection on characters possessing low heritability, and especially so if their environmental correlation with other desirable characters of higher heritability is high, while the genetic correlation is low or negative. An illustration of such a situation is provided by Hazel's (1943) study of selection indexes in swine. One of the index components, score for market suitability, makes a positive contribution to the total economic value. It has a fairly high heritability, but its environmental correlation with the more important economic trait, growth rate, is higher than its genetic correlation. Consequently, an index in which market score is given negative weighting is more efficient than one where it is ignored. A positive weighting for market score, which would enter an index constructed without reference to the correlations between component traits, would hinder progress even more. It should also be noted, as has been pointed out by others, that the rate of change of a

particular character may, due to its negative genetic correlation with some other character of greater heritability or of greater importance, be opposite to the direct effect of selection on this character. This phenomenon may be readily illustrated by another example based on data obtained in a laboratory.

Let us assume that, in the case of New Hampshire chickens, long keels are desirable, while long shanks are not. For the sake of simplicity we may also assume that fitness is completely determined by these two traits. Furthermore, let us postulate that the contribution of keel length to hypothetical fitness is half that of shank length. The phenotypic aggregate, which is the selection index (I), is then equal to $K - 2S$, where K stands for keel length, and S for shank length. Selection presumably then should increase the length of the keel, while reducing the length of the shank.

Using the data from the report of Lerner, Asmundson and Cruden (1947) the expected changes in these characters may be computed for a population of chickens selected for the short-shank—long-keel complex. Figure 1 illustrates the interrelationships between these traits. The methods of computation are based on Wright's path coefficient technique, as elaborated by Hazel (1943) and by Dempster and Lerner (1947). As shown in the column headed "natural index" in table 2 selection of the type postulated will lead to a reduction in both shank length and keel length, in spite of the predicated advantages of longer keels. It is then clear that the direction of selection pressure does not necessarily coincide with the direction of observed changes in a population. The obvious temptation to identify the observed changes of an individual character in a natural population with selection intensity applied to it should be guarded against.

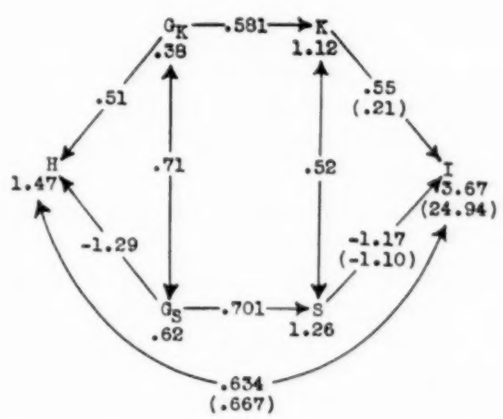

FIG. 1. Interrelationships between the phenotypes of shank length (S), keel length (K), the genotypes for S (G_S), for K (G_K), the selection index (I), and the aggregate genotype for the short-shank—long-keel complex (H). Arrows indicate path coefficients; double headed arrows—correlations. The figures below each symbol show the variances. The figures in parentheses show the altered values when the "ideal" index is used instead of the postulated "natural" index.

TABLE 2. *Rates of change per generation per standard deviation of the selection differential under the "natural" and the "ideal" indexes*

Selection index (I)	Natural index Keel length minus twice the shank length ($K - 2S$)	Ideal index Keel length minus five times the shank length ($K - 5S$)
Change in aggregate genotype (ΔH)	.769	.809
Change in the average genotype for keel length (ΔG_K)	−.161	−.260
Change in the average genotype for shank length (ΔG_S)	−.465	−.534

The previously noted point, that the index in which the components are weighted according to their postulated contribution to fitness (the "natural index") is not as efficient an index as that which takes into account the heritabilities and genetic correlations of the traits entering into it, can be readily demonstrated from the same data. By the use of Hazel's method for constructing selection indexes the "ideal index" (one leading to the maximum rate of progress with respect to H, the aggregate genotype of the shank-keel complex) was found to equal keel length

minus five times shank length, rather than minus twice the shank length. The rate of change in H under the natural index is .769/.809 or about 95 per cent as great as it is under the ideal index. It is also true in this case that the direction of change in keel length is reversed. Were the genetic variance of keel length considerably greater, both the natural and the ideal indexes would lead to changes in both components in the direction desirable for each. Under other conditions, particularly where the heritability of an important character is very low and the environmental correlation between it and another trait with greater heritability is high, the difference in efficiency between the natural and the ideal indexes can be much more marked.

Fundamental Theorem of Natural Selection

If selection can lead to changes in a given character in the direction contrary to that of selection pressure, it may at first sight appear possible that a decrease in genetic fitness itself may ensue if the components of the index for fitness are weighted solely for the amounts of their contribution to the phenotypic aggregate without regard to their heritabilities or to their interrelations. If this were the case an index operating in nature could in the extreme lead to the extinction of a population. However, Fisher (1930, p. 35) in his fundamental theorem of natural selection (see below) has shown that selection pressure will always tend to increase fitness (although this does not imply that fitness, as defined, necessarily improves the competitive ability of a population with respect to other populations not interbreeding with it; see Haldane's [1932, pp. 207–210] discussion of this point). Further consideration of the path diagram in figure 1 leads to the same conclusion. Let us assume in this instance that two characters (S and K in this example) are expressed in units which make their contribution to the index I numerically equal. The rate of change in the genetic aggregate of fitness, or ΔH, is then equal to $\Delta G_K + \Delta G_S$, where as before G_K and G_S are the genotypes for keel and for shank respectively. From the path relationships in figure 1 this can be rewritten in the form

$$\Delta H = \bar{\imath}\left[\frac{\sigma_{G_K}}{\sigma_I}(h_K\sigma_K + h_S\sigma_S r_{G_K G_S}) + \frac{\sigma_{G_S}}{\sigma_I}(h_S\sigma_S + h_K\sigma_K r_{G_K G_S})\right],$$

where $\bar{\imath}$ is the intensity of selection in standard deviations, and I again the selection index. This equation (since $i/\sigma_I = \bar{\imath}$) readily reduces itself to

$$\Delta H = \frac{i}{\sigma_I^2}[\sigma^2_{G_K} + \sigma^2_{G_S} + 2\sigma_{G_K}\sigma_{G_S} r_{G_K G_S}].$$

The expression in brackets is a perfect square, because it is equal to $\sigma^2_{G_K+G_S}$. Consequently, ΔH cannot be negative. In effect the above equation is Fisher's fundamental theorem derived by Wright's path coefficient technique for the case where additive effects of gene differences predominate (see also Wright 1935b). The exact statement of Fisher's theorem is "The rate of increase of fitness of any organism at any time is equal to its genetic variance in fitness at that time." If the term i/σ_I is transferred to the left side of the first of the above equations, $\Delta H\sigma_I/i$ will represent the rate of change in H per standard deviation in the selection differential, while the right side by definition is the genetic variance of fitness.

A further transformation may be made here by considering the fact that the expression in the brackets is actually equivalent to $\sigma^2_{G_I}$. Hence,

$$\Delta H = \frac{i}{\sigma_I^2}\sigma^2_{G_I} = \frac{i}{\sigma_I^2} h_I^2\sigma_I^2 = ih_I^2.$$

This is the same expression which was previously derived for a single character X. It is evident that fitness or aggregate genotypic value may be treated as a single character with its own heritability. However, the correlation between an ideal index and fitness is greater than that be-

tween the phenotypic and genetic aspects of fitness.

Sequential Selection

The effects of selection for fitness in relation to the component traits are rendered more complex by the fact that adaptive characters may be subjected to selection pressures at different stages of the life cycle. This type of selection may be termed, in accordance with modern statistical usage, *sequential selection*. The advantage of one competing type over another is not necessarily expressed in terms of an index in which all components exercise their effects concurrently. For instance, the number of eggs produced, fertility of these eggs, the hatchability of the fertile eggs, neo-natal mortality, infant mortality, adolescent mortality, and rate of sexual maturity form a sequence of factors each one of which changes the composition of the original population. If the original population was normally distributed for the genetic aggregate of fitness, the first selection will lead to a truncation of the distribution curve. The second selection operates on a new distribution, as does each subsequent one. The selective advantages of one type over another at each stage will depend on what happened under the previous selection. Hence, the statistical model which is used in the computation of selection intensity on the basis of gross fitness indexes may not be strictly applicable, and selection intensities estimated in such manner not entirely accurate. The development of the techniques of sequential analysis will probably permit more valid estimates to be made in the future, but only if the stages at which selection operates are identified experimentally.

As an example of application of the situation described to breeding practice our work on the optimum age composition of poultry breeding flocks (Dempster and Lerner, 1947, fig. 3) may be cited. Among several cases considered, computations were made for a case in which birds were selected on the basis of an index combining their own annual egg production records with those of their sisters. The expected genetic gain was found to be 32.7 per cent of the maximum gain that would be possible, were the actual genotypes of the birds known. In other words, selection based on the optimum combination of phenotypic measurements of the birds themselves and of their sisters is only .327 times as efficient as that possible in the hypothetical case of selection on the basis of actual genotypes. Now it is possible to restrict the population in question by subjecting it to a previous selection based on a similar index for the early part of the year's record rather than for the complete annual production record as in the first instance. The result of this restriction is that the percentage of the maximum hypothetical gain possible which is realized, when the restricted population is selected from on the same basis as in the first case, is now reduced to 24.7. Furthermore, the maximum possible gain is itself decreased due to a reduction in the genetic variance produced by the first selection. Thus, it may be seen that truncation of the distribution curve of a population at one stage of the life cycle can affect materially the amount of genetic change produced by selection operating at a later stage.

Adaptive Value of Additive Gene Action

From all of the above considerations it is clear that the identification of the stages at which natural selection operates, and, indeed, some estimation of the characters which enter a fitness index is an essential step in evolutionary studies. A difficulty to add to the complex situation described, is that certain traits may contribute to fitness only indirectly through their effect on adaptiveness (Darlington, 1939). Selection for devices to alter mutation rates (Sturtevant, 1937), to regulate the amount of cross-fertilization in plants (Mather, 1943), to alter the frequency of chiasma formation (Stebbins et al., 1946), or to increase high environ-

mental variability where selective pressures are high (Haldane, 1932, p. 177) may all be cited as instances of this kind. Another factor of a similar nature may be suggested in a possible selection for additively acting genes. Such genes would lead to higher degrees of heritability than genes with largely non-additive effects (effects of gene differences exhibiting complete dominance are in the main additive, except in cases of exceeding rarity of the recessive alleles). In turn, higher heritabilities confer an advantage on a group in competition with another group, because an equal selection intensity will allow more rapid changes in the former and thus increase its plasticity. This may constitute a partial explanation for the fact that few non-additive deviations have been found in such characters as sexual maturity and body weight in chickens (Lerner, 1945; Hazel and Lamoreux, 1947), milk and butterfat yield in cattle (Seath and Lush, 1940), and yield in crosses between non-selected lines of corn (Sprague and Tatum, 1942). A somewhat different argument for the selective advantages of additive genes has been previously advanced by Seath and Lush (1940) who pointed to the selective disadvantages of genes producing favorable results in combination with rarely occurring genetic backgrounds and unfavorable results in most combinations.

Summary

The relative number of offspring which an individual leaves or, in other words, the individual's fitness depends on its phenotype for the characters on which natural selection operates. If the phenotype is perfectly correlated with the genotype, that is to say, is completely determined by it, then the changes in the average genetic constitution of a population with respect to the character concerned will correspond in direction and magnitude to the selection pressure applied to the phenotype. However, particularly in the case of metrical characters, this correlation is far from perfect. Hence, a given selection pressure on the phenotypes is not necessarily reflected fully by the changes in the genotypes (and hence phenotypes) in the following generation. Workers in the field of animal breeding have elaborated statistical techniques for measuring the degree of determination of phenotypes by additively acting gene differences or, as this statistic is known, the degree of heritability. The use of such techniques permits the computation of expected changes in the average genotype of a population subjected to selection. For any given selection pressure the rate of this change per unit of phenotypic variance of the trait under selection is actually directly proportional to its heritability.

When selection is applied not to a single character but to a complex of traits, the rate of change in the aggregate genotype depends not only on the contribution that each trait makes to the aggregate, but also on the respective heritability of each component, and on the extent to which the various traits are correlated with each other. Such correlations may be either genetic (due to pleiotropy, linkage or common origin of the gene complexes for the correlated traits) or environmental (due to the common environment under which these traits develop). The consequences of the two types of correlation with respect to the effects of selection are radically different. Methods for separating genetic from environmental correlations have been developed, and are in use in the field of animal breeding.

These methods permit the construction of selection indexes which lead to greater efficiency than is possible when selection is based on phenotypic relations only, as it is in nature. Natural selection cannot take account of the relative heritabilities of various traits nor of the correlations between them, and is, therefore, in this sense somewhat inefficient. Illustrations have been given of cases where, because of the type of genetic correlation and the degree of heritability of the component traits of an aggregate, changes

with respect to a character in a population may occur in the direction opposite to that of the selection pressure applied. It follows that evidence of temporal changes in natural populations does not provide an adequate measure of the magnitude or even direction of the selection pressure. It is also demonstrated that reversals of the type referred to above are possible only for the components of an aggregate, but not for the aggregate itself. The ultimate complex of traits in nature is fitness, and, as Fisher has already shown, selection pressure will always increase fitness.

A further point is brought out in connection with selection for an aggregate of traits, which deals with the problem of sequential selection. This occurs when selection pressure is applied to different component traits at different stages of the life cycle. Special types of statistical analyses are called for in such cases. An example from the field of poultry breeding is given to illustrate the effects on the expected genetic changes, of selection applied to a population already restricted by a previous selection.

From the illustrations cited, it may be seen that the type of investigation currently carried on by students of animal breeding may offer useful evidence and techniques to students of evolution. It must be admitted that there are differences between artificial and natural selection which would introduce difficulties in the use of animal breeding methods for the study of the latter. However, the techniques developed for the simpler situation can be useful as a stepping stone to an understanding of the much more complex situations found in nature. From studies on artificial selection of domestic animals it appears that collection of data for the tentative construction of selection indexes is one of the necessary approaches to future studies on natural selection. Only some knowledge of the characters, their contribution to fitness, the stages at which the selection operates on them, their heritabilities, and genetic correlations between them, will permit approximate determinations of the magnitude and direction of selection pressures in nature.

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ONTOGENETIC EVOLUTION IN FROGS

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Received January 5, 1948

The study of evolution in the ontogeny of the Frogs (Order Salientia or Anura) has been rather neglected. They have probably been dismissed as one of the minor orders of the vertebrate phylum, characterized by a well-known life cycle, which comprises an aquatic larval period and metamorphosis into terrestrial tetrapods. The tropical and subtropical frogs show many deviations from this mode of development. At the present time, the life histories of less than one-third of the described species of frogs are known (Orton, 1946). It seems probable that as the opening up of this field proceeds other unusual life histories will be added to those already described in the much scattered literature. The very facts that the Amphibia are the most complex vertebrates in which larvae occur, that those of the Salientia are not always aquatic and are sometimes replaced by direct development, ought to make the order interesting from the point of view of ontogenetic evolution.

The present preliminary survey is based on a number of peculiar modes of anuran development observed in southeastern Brazil, in the course of many years, by the late Professor Adolpho Lutz, the author, and their assistant Mr. Joaquim Venancio, and recorded either for the first time (Lutz, 1928, 1929, 1931; B. Lutz, 1944; Lynn and B. Lutz, 1946, 1947), or in confirmation of earlier work. Parallel or convergent cases from other regions may be mentioned but the data available do not permit detailed comparison. (For factual literature see Brandes and Schoenichen, 1901; Noble, 1925, 1927, 1931; B. Lutz, 1947).

GENERAL TREND

The general trend of modified salientian ontogeny is towards withdrawal of development from water. Modifications of reproductive behavior, habitat preferences and ecotopic adaptations of the adults are correlated with it. The most important morphological change is the increase of yolk reserves. Acceleration of development also occurs.

Most of the changes observed are caenogenetic. They affect the embryos and sometimes the larvae and are already indicated by the size of the unfertilized ovum. It seems best to include them in the general concept of *clandestine evolution* (De Beer, 1930), which must have a very wide field of application in embryology. Such changes cannot be registered by paleontology and are accessible to comparative anatomy only during a transient phase. As long as anuran taxonomy continues to be based exclusively on adult morphology they will be left aside in establishing phylogenetical relationships.

Some of the novelties are monophyletic. Others recur in different regions under similar ecological conditions and, with variations as to detail, in groups considered as phylogenetically distinct. Where instances of parallel or convergent evolution appear to be absent this may be due to the lack of similar ecotopes [1] or, more likely, to their not having been investigated. It is, however, only fair to state that the present systematics of Salientia is artificial and in need of revision.

[1] Ecotope = ecological niche; ecotopic = relating to an ecotope. Ed.

TABLE 1. *Comparative table of development*

Name	Placement of eggs	Stage at hatching	Subsequent development
HYLIDAE			
Hyla decipiens	On open leaves	Rudimentary larva	Aquatic
Centrolenella	On open leaves	Fully formed larva	Aquatic
Phyllomedusa	Leaves folded over the clutch	Fully formed larva	Aquatic
Coelonotous Hylidae	Back of ♀ (in single mass)	Submetamorphic larva	Aquatic (in adult habitat)
PIPIDAE			
Pipa	Back of ♀ (in individual chambers)	Adult form	Simple growth
BRACHYCEPHALIDAE			
Rhinoderma darwinii	Vocal sac of ♂	Adult form	Simple growth
BUFONIDAE (sensu stricto)			
Cyclorhamphus fuliginosus, *Cyclorhamphus pinderi*	Wet ledges of rock	Larva	Semi-aquatic (gliding on rocks)
Thoropa petropolitana, *Thoropa miliaris*	Wet outcrops of rock	Larva	Semi-aquatic (gliding on rocks)
Zachaenus parvulus	Hollows in banks	Terrestrial larva	Terrestrial: In hollow
LEPTODACTYLIDAE			
Eupemphix, *Physalaemus*	Edges of pools	Rudimentary larva	Aquatic
Leptodactylus flavopictus, *Leptodactylus pentadactylus* (Brazilian form)	Pot-shaped holes	Larva	Aquatic
Leptodactylus mystaceus	Earthen nests open below	Larva	Aquatic (semi-permanent water or damp spots)
Leptodactylus nanus	Closed earthen nests with aperture in roof	Terrestrial larva	Terrestrial (in nest)
BUFONIDAE (sensu lato)			
Eleutherodactylus	Varied: Hollows, Bromeliads, etc.	Adult form	Simple growth

Withdrawal of development from water is achieved by diverse devices and to varying degrees. In some cases special types of aquatic or of damp environments are utilized. In other cases water is altogether avoided. The alternative sites for placement of the eggs and ontogeny are quite varied but may be reduced to a few main categories as shown in table 1.

Organic Selection

The modifications of reproductive behavior and the habitat preferences of the adults obviously fall within the scope of organic selection, i.e. selection of the environment by the organism (Baldwin, 1896; C. Lloyd-Morgan, 1900, as cited by J. Huxley, 1942). To take but a few examples: the genus *Cyclorhamphus* has become closely adapted to a troglodytic way of life but other neotropical frogs, like some species of the diurnal, running water genus *Elosia*, seek shelter in grottos at night, and the extremely arboreal *Hyla albofrenata* is occasionally heard calling in crevices. Spawning on leaves by Hylidae is merely an omission of the usual descent into water for this purpose; the reason for it is readily apparent in *Phyllomedusa*, which has no webs between the digits and avoids swimming.

Changes of reproductive behavior and habitat preferences merge into ecotopic adaptations by selection in the usual sense.

SELECTION PRESSURE

The withdrawal of development from water and the correlated ecotopic adaptations of the adults have an interesting feature in common, in regard to selection pressure.

Adult Salientia are preyed upon by arboreal snakes, birds, a few mammals and larger members of their own order and sometimes of their own genus and species. They are attacked by leeches, mites, ticks and mosquitoes. They have evolved various devices against predators, such as concealing or aposematic coloration, protective resemblance to their backgrounds (Cott, 1940), nocturnal habits and the choice of inaccessible habitats (tubular bromeliads and bamboos, burrows and crevices, etc.).

Interspecific competition is minimized by ecotopic divergence both of adults and of tadpoles.

The enemies of larval salientians are not the same as those of the adults. With the possible exception of aquatic birds and snakes, fish and a few fresh water invertebrates, the chief predators on tadpoles are aquatic insects (see table 2).

In Brazil, at the time of spawning numerous species of frogs congregate at most still waters, especially the ponds and lagoons of the more arid sections of the country. There, the beginning of the rainy season coincides with flooding and with the onset of the breeding period of the anurans. Available habitats are less varied than in the rain and mist forest. Many tadpoles have to compete with each other for food and are exposed to predation. Such waters have populations of well-armored insects, the carnivorous habits of which can be observed in nature and are described in the literature (see Needham and Lloyd, 1916 and Costa Lima, 1939–43). Where predatory insects are abundant there is generally a striking dearth of tadpoles, especially small ones. Other kinds of enemies, if present, especially the water-fowl, may also take their toll. Under these circumstances spawn has to be abundant to insure survival Eggs are very small and numerous, ranging from hundreds to thousands.

In the mountain streams of southeastern Brazil, the number of species of tadpoles is very much reduced and those present resort to special niches. Some (*Centrolenella eurygnatha*) hide under dead leaves and other detritus which accumulates in the still reaches. Others (*Phyllomedusa guttata*) float in schools, breaking the surface with their enlarged dorsal mouth-funnel. A few *Hyla* tadpoles cling with their lips and teeth to the rocks. Those of *Elosia* hide between stones at the bottom. Delayed hatching, as in the first two species mentioned, provides additional protection against aquatic insects, which are generally not so large as those of the still waters. Fish are mostly absent or rare and small if pres-

TABLE 2. *Insect enemies of tadpoles in S.E. Brazil*

Taxonomic groups	Habitats	Abundance	Stages	Feeding habits
PLECOPTERA	Mountain streams	Common	Nymphs	Carnivorous
ODONATA	Still water bromeliads	Very common Common	Naiads	Carnivorous; have been reared on tadpoles
TRICHOPTERA	Mostly mountain streams in S.E. Brazil	Very common	Larvae	Herbivorous, occasionally carnivorous; once seen attacking a small tadpole in laboratory
HEMIPTERA				
Nepoidea:				
(a) Belostomatidae:				
Lethocerus (giant forms; may exceed 100 mm.)	Still waters	Very abundant	Nymphs and imagines	Attacks tadpoles
Belostoma	Still waters	Very abundant	Nymphs and imagines	Attacks tadpoles
(b) Naucoridae:				
Cryphocricus	Mountain streams (still reaches)	Rather rare	Nymphs and imagines	Predatory
(c) Nepidae:				
Ranatra	Still water and marshes	Not very common	Nymphs and imagines	Predatory
COLEOPTERA				
Diving Beetles:				
(a) Hydrophylidae (b) Dytiscidae	Still water	Swarms	Larvae and imagines	Carnivorous. Larvae very predatory; an adult seen attacking a *Phyllomedusa* tadpole
DIPTERA				
(a) Tabanidae	Bromeliads	Some species common in certain locations	Larvae	Carnivorous
(b) Syrphidae:	Bromeliads	Some species common in certain locations	Larvae	Carnivorous
Rhysops berthae	?	Fairly common in certain locations	Larvae	Parasitic on eggs of *Centrolenel.a eurygnatha*

ent. Kingfishers are sometimes seen flying near the banks of streams and, occasionally, water-ouzels are observed on them.

Bromeliads contain many forms of animal life, including small snakes, lizards, crustaceans, mollusks, spiders, occasionally leeches and scorpions and many insects. The possibilities of hiding are, however, much increased by the division of the leaf cup into a central tube and outer compartments, narrowing towards the base of the rosette of leaves, inserted in a very low spiral. The prolonged carrying by the mother of the eggs on her back extends the protection of the adults to their progeny, and the shortening of the aquatic period to a few days, at submetamorphosis, decreases the risk from predation and also from drying, during prolonged rainless periods. A few other bromeliad-dwelling frogs with ordinary tadpoles spawn in the leaf cups but they are very small species and lay only a few eggs. The coelonotous Hylidae need not compete for food with other larvae since they hatch with abundant yolk-reserves.

There are no competing species in the bamboo in which we found the coelonotous *Hyla ohausi*. The other inhabitants are coleopteran maggots, presumably vegetarian, and culicid larvae, in regard to which the predator-prey relationship is to the advantage of the frogs. Woodpeckers and monkeys probably hunt for the beetle grubs but small, dark, submetamorphic anurans, unable to leap out, may find a hiding-place in the black water and rich mulch which accumulate in the hollows between the septa of the perforated bamboos.

The semiaquatic tadpoles of the troglodytic *Cyclorhamphus* and the nonaquatic larvae of the rock-dwelling *Thoropa* are unmolested by aquatic forms of life. Small insects have been seen, by Mr. Venancio, hovering over the spawn of *C. pinderi,* lying on a ledge of rock in a grotto, but they were being snapped up by the adult posted in the vicinity. Both adults and larvae of *Cyclorhamphus* have concealing coloration and those of *Thoropa* are protected by their resemblance to the wet outcrops of rock on which they live. The dimensions of their habitats are ample enough to make interspecific competition unnecessary. *T. miliaris* is large and has more roaming habits than *T. petropolitana. Cyclorhamphus fuliginosus* and *C. pinderi* are allopatric forms (Mayr, 1942).

The leptodactylids that produce froth provide a good example of the gradual elimination of predators and competitors as specialization in the site of spawning progresses. The small genera *Eupemphix* and *Physalaemus* lay their eggs at the edge of very small puddles and pools and are exposed to the usual hazards. The spawn is abundant and the larvae hatch in a very rudimentary condition; during the first days they live under the froth or return to it for shelter. The nests of *Leptodactylus mystaceus,* which are open below, communicate with semipermanent waters but entrance into them can be retarded. The pot-shaped spawning holes of the large species of *Leptodactylus,* like *flavopictus* observed by us, are covered by a deep blanket of froth that keeps out competitors and marauders. Almost inviolable isolation is achieved in the earthen pans of *L. nanus* which are distant from water and can be entered or left only by a small aperture in the built-up roof.

Under these circumstances, it seems valid to state that withdrawal of development from water reduces selection pressure during ontogeny. The consequences of this fact, as will be shown later, are very important in the ontogenetic evolution of the terrestrial vertebrates.

Nonaquatic development cannot, of course, preclude the appearance of new enemies, such as the syrphid fly, *Rhysops berthae* Costa Lima (1946), which is parasitic on the eggs both of *Centrolenella eurygnatha,* and of a rhyacophilid caddisfly which also spawns on open leaves above mountain brooks. The formation of mildew on nonaquatic eggs of *Eleu-*

therodactylus, observed by Ruthven (1915) and by B. Lutz (1948), may also come under this heading. It is, however, not yet clear whether fungus infestation occurs in nature as well as in the laboratory.

The rôle of intraspecific competition during development, stressed by Haldane (1932), for intrauterine ontogeny, is less obvious. Increased yolk reserves, coupled with intraoval development, make for independence of the embryo from its siblings. Slight differential factors may ensue from the relative position of each egg in the clutch. They can hardly go much beyond the possible advantage of a central over a peripheral position in clutches disposed in one layer. In those laid in several layers greater differences may obtain as to gaseous exchanges with the outside. Competition among terrestrial, sedentary larvae nourished on yolk is evidently far less than it is among free swimming tadpoles, which have to secure their own food.

The Importance of Large-Yolked Eggs

The passage from microlecithal (small-yolked) to macrolecithal (large-yolked) eggs is the most important aspect of the ontogenetic evolution of the Salientia. Incidentally, it sheds some light on the evolution of the next higher classes of the vertebrate phylum.

There is no literature on tropical frogs comparable to the synopses of the Wrights on the eggs and on the tadpoles of North American anurans, nor are there enough figures available for statistical treatment. The cases in which spawning was observed or in which the eggs were measured and counted are used in table 3. The examples are few and somewhat heterogeneous but they show the general trend.

Given the usually very small size of the eggs of frogs and the relative size of the mothers, the increase in volume of yolk is significant. Moreover, it is accompanied by a drastic reduction in the number of eggs to a small fraction of the complements generally laid. The criteria by which it must be judged are the lengthening of intraoval life which increased yolk reserves make possible and the degree of development attained without other sources of nourishment. In many cases the reserves are sufficient to permit reaching adult shape with remnants of yolk in the gut, thus allowing for a short period of adaptation to the new environment before the risks involved in hunting need be incurred.

Noble, who did outstanding work on the biology of the Amphibia (1925, 1927, 1931), believed that increase of yolk might be random and occur by sudden change. He mentions the fact that in *Gastrotheca* the eggs may be either large

Table 3. *Egg size and egg number in Brazilian frogs*

Species and size of female (snout to vent)	Diameter of yolk (in mm.)	Cubage of yolk (cub.mm.)	Intraoval development (in days)	Egg complement	Site of eggs
1. *Physalaemus fuscomaculatus* 40 mm.	1.0	0.52	1	1000	standing water
Hyla polytaenia 38 mm.				200	
2. *Hyla mesophaea* 75 mm.	1.5	1.77	1	1000	leaves
3. *Phyllomedusa guttata* 46 mm.	2.0	4.19	14	49	
Centrolenella eurygnatha 26 mm.				10–30	
4. *Leptodactylus nanus* 24 mm.	2.5	8.18	?	8–10	closed pans
5. *Coelonotus fissilis* 36 mm.	3.0	14.14	14–21	7–13	back of the ♀
6. *Hyla goeldii* 38 mm.	3.5	22.45	14–21	25	
7. *Eleutherodactylus nasutus* 38 mm.	4.0	33.51	28–30	13–14	bromeliads
8. *Eleutherodactylus guentheri* 40 mm.	4.5	47.71	30–35	22–25	banks of earth

or small. This point of view may have been induced by the fact that in frog ovaries not all the ova reach full size simultaneously. In developing clutches of large-yolked eggs there are often a few small ones which do not develop. Examination of the ovaries of adult *Phyllomedusa* or *Eleutherodactylus* females shows the presence of large-yolked and of small eggs. In the genus *Phyllomedusa* the small eggs, or yolkless egg membranes, are found glueing together the edges of the leaves folded over the egg mass by the parents while spawning.

As those Amphibia that were ancestral to the Reptiles may have had large-yolked eggs, Noble (1931) suggests that microlecithal eggs may not be necessarily more primitive. The comparative study of modern tropical anuran development certainly bears out the probability that the Amphibia ancestral to the Reptilia were macrolecithal, but it also permits interpreting small-yolked anuran eggs as relict. The evolutionary value of macrolecithal eggs is shown by the fact that large-yolked eggs are the rule in terrestrial vertebrates, until the viviparous mammals are reached. Irrespective of the enzymes that appear at hatching-time, it is quite obvious that when the possibilities of development, within the egg membranes, or at the expense of stored yolk, are exhausted the young organism must rely on outside sources of nourishment. The more undeveloped it is when this stage is reached, the more defenseless will it be in the struggle for survival. In the poikilothermal (ectothermal) vertebrates with increasingly macrolecithal eggs this moment is progressively put off, towards the end of development (anurans with modified ontogeny), or by hatching in adult shape (anurans with direct development, reptiles). The homoiothermal (endothermal) birds, which have helpless chicks, resort to feeding them. The increase of yolk reserves to the limit attainable for each kind is the first step towards better provision for the young common to all classes of oviparous vertebrates.

Extent of Morphological Change

The extent of morphological change is in agreement with the size of the yolk. The resultant mode of development follows two main trends, *repetition* and *compensation*. One or the other predominates, according to whether ontogeny is larval or embryonic.

In the former case, there is a repetition of the life cycle of larva, metamorphosis and imago, though hatching is delayed either to the secondary, perfect tadpole stage, without external gills or limbs (*Centrolenella, Phyllomedusa*), or to submetamorphosis (coelonotous Hylidae). When the larvae are adapted to a free-swimming or to a semiaquatic existence, they show minor morphological changes, fitting them for life in their respective environments. Those living on rocks for instance, like some species of *Cyclorhamphus* and *Toropa*, are streamlined with narrow tails and clinging mouths. In nonadaptive, sedentary, terrestrial larvae, nonfunctional organs such as the fins and horny teeth are reduced.

The external gills are more variable. They may have the usual form (*Centrolenella, Phyllomedusa*) or be enlarged. In the coelonotous Hylidae they are bell-shaped; in both cases they occur in the encapsuled stage and regress as or before the larva emerges from the eggs. Some free, terrestrial tadpoles (*L. nanus*) show rudimentary gills for a short period. The complete absence of external branchiae is not uncommon in anurans developing from nonaquatic eggs (Lynn, 1942).

In macrolecithal eggs cleavage is retarded; the early embryo develops on top of the yolk and forms a precocious network of blood vessels over it. This marks a transition to the conditions found in the frogs which hatch in adult shape.

The development of these is embryonic and there is no metamorphosis. The changes of structure are correspondingly greater. Some species of *Eleutherodac-*

tylus from the Caribbean region develop external gills for a while but in others, including those from southeastern Brazil studied by Lynn and B. Lutz (1946, 1947), the pharyngeal pouches never break through and there are no open gill slits. The development of functional internal gills is therefore impossible and there are no external ones. The operculum and the typical tadpole mouth, with its supporting cartilages, are absent. The intestine though convoluted is not spirally coiled as in tadpoles. Both pairs of limbs develop outside and the limb buds appear quite early, thus obliterating one of the distinctions between Amphibia and Amniota.

The larval tail of *Eleutherodactylus,* however, persists and develops further. It passes between the legs of the imprisoned embryo and becomes ventral, with the parts corresponding to the upper and lower fins in a lateral position. The membranous surface is greatly increased and is closely applied to the egg membranes; it is intensely vascularized and active circulation can be observed in it. The tail, having lost its locomotory function, has become the main respiratory organ.

The process by which this occurs is one of compensation. It is akin, but not identical, with the "substitution" of Kleinenberg (1886) by which one organ serves as a scaffold and its presence as a stimulus for the formation of another, to wit the rôle of the notochord in the development of the backbone. The usual cutaneous gas exchange of the tadpole tail, no doubt, provides the initial possibility for its transformation into a respiratory organ. However, by becoming one, the tail compensates for the anuran larval gills, which are no longer present, and for the respiratory function of the sauropsid allantois, which has not yet evolved. Compensation is thus seen to be a further diversification of an organ for the accomplishment of a function for which a special organ is lacking.

Respiratory tails are known in other anurans with direct development, including the recently described embryo of the Ceylonese *Rhacophorus ? reticulatus* (Kirtisinghe, 1946), which develops from terrestrial eggs.

Acceleration and Its Consequences

A number of interesting facts connected with the rate of development come to light as a result of studies on modified anuran ontogeny.

Haldane (1932) points out that there has been a common tendency in evolution toward the acceleration of development, i.e. for certain characters to appear progressively earlier in the life cycle and, conversely, another tendency towards retardation.

Now, in animals with distinct larval and adult stages that live in different environments, such as the Salientia with aquatic larvae and terrestrial adults, adaptive requirements are evidently different for each of the two stages. Moreover, the transition from one stage and environment to the other requires considerable organic remodelling.

This is a very important point. In the phylogenetic evolution of the terrestrial vertebrates there comes a time when larvae no longer occur and costly metamorphosis is done away with. That there has been a break is evident in the Sauropsida. I interpret nonaquatic anuran ontogeny as equivalent to a preliminary phase of this evolutionary process. With increasing yolk reserves, there is first a reduction, next a suppression of free-swimming larval life and, finally, an abolition of the larval stage.

Some forms with terrestrial development (for example, *Leptodactylus nanus* and *Zachaenus parvulus*), it may be objected, retain tadpoles; no doubt, but these tadpoles are sedentary and nourished exclusively on yolk. They and, *a fortiori,* the embryos of frogs with direct development (*Eleutherodactylus* and others) come under the heading of sheltered embryos (or larvae), in which form is independent of survival value as sug-

gested by Haldane (1933). This has far reaching consequences. The need for dual adaptation to two separate environments is done away with. Development becomes self-contained and there is an abbreviation of the formative process, which follows the shortest route from fertilized ovum to adult shape.

Acceleration in itself is not an abstract concept, nor yet a cause. It is merely an effect of morpho-physiological changes in secondary egg structures and during development. Nevertheless, it has led to the suppression of the larva and of metamorphosis in the life cycle of the terrestrial vertebrates. Regarded thus, its phylogenetical value and its evolutionary significance become clear.

Obstacles to Further Evolution

One may ask why modern Salientia having evolved thus far were unable to continue evolving. This leads to the discussion of the obstacles in their way. These are generally considered to be the difficulty of emancipating the young from water and the moisture requirements of the adults. Some slight changes in this concept may lead to a more precise definition.

The examples used here show that the difficulty of emancipating the young from water, as an *outside environment,* is not insuperable. It has been overcome a number of times and in diverse manners. Development is admittedly not as well isolated from contact with the outside as in the cleidoic eggs of reptiles and birds. Nor is this necessary, provided that the physiological requirements of the embryos are fulfilled. In some ways they are easier to provide for than those of embryos better protected by hard shells against evaporation and other environmental hazards.

Yolk provides the necessary food in both groups. Water metabolism may be attended to by the intraoval fluid and the gelatinous membranes of anuran eggs which take up moisture very readily. The membranes of those which are buried are relatively thick. Evaporation is guarded against in all the nonaquatic anuran eggs mentioned by the damp spawning sites, or by the more or less saturated condition of the whole environment, especially in the rain and mist forest where most of these life histories occur. Respiration of salientian embryos can take place through the egg membranes. The morphological nature of the respiratory organs (superficial network of vessels, gills, or respiratory tails) is not of paramount importance. The main point is that gaseous exchange be possible and that the supply of oxygen be sufficient. If this is assured it seems somewhat immaterial whether the outside environment be aquatic or not. Nonaquatic embryos of *Eleutherodactylus* can be kept alive in water if the thick egg membranes are removed. Developing eggs of *Centrolenella eurygnatha,* which drop into water from the supporting leaf, survive as long as intense aeration is assured.

Mutatis mutandis, this also applies to tadpoles. *Phyllomedusa guttata* larvae, which are surface swimmers, have been kept alive by A. Lutz and me on water ferns (*Hymenophyllum*) for over an hour at a time and then returned to water. The tadpoles of *Leptodactylus mystaceus,* which spawns in places likely to be flooded, have been seen in damp locations by Hensel (1867) and others since. The average aquatic tadpoles develop lungs early and come up to the surface periodically. Only a few species, like *Hyla claresignata,* remain entirely submerged, clinging to boulders in swift flowing and torrential waters.

The moisture requirements of the adults are due to the persistence of the dual respiratory mechanism already seen in the tadpoles. Breathing is both cutaneous and pulmonary, with buccopharyngeal respiration in subordinate rôle.

Some adult frogs are almost completely aquatic. This is the case with the rather primitive, aglossal African *Xenopus,* with the Neotropical *Pipa,* and also with the

liopelmid *Ascaphus truei* from the cold and swift mountain streams of the northwestern United States, studied by Helen T. Gaige (1920). The lungs of the latter were investigated by Noble (1927) and found to be poorly developed. Conversely, large terrestrial toads of the genus *Bufo* are less dependent on moisture than most other Salientia. Marcus (1927) cited by Noble (1931) points out that the lungs of the large neotropical species are very well developed and vascularized. The skin is thick and glandular. We find that forms from the semiarid sections of Brazil, like *Bufo paracnemis* Lutz, have extra glands on the legs. These are found again in *Bufo alvarius* from similar regions of North America and in *Odontophrynus cultripes* from the scrub section of central Brazil.

The correlation seems evident. As long as the lungs are poorly developed, the skin has to be used as a respiratory organ and it can only function as such by remaining moist and permeable, allowing gases to enter into solution so that they can pass into the blood stream or be expelled from the body.

It is not the dependence of early life on an *aquatic outside* environment which constitutes an insuperable barrier to the further evolution of the Salientia. The real obstacle consists in the dual respiratory mechanism, or, to be more precise, in the lack of its concentration in one, well-developed organ, the lung.

Highest Evolutionary Level Reached

Comparison of some species of the two neotropical genera, which have evolved furthest as to the mode of development or as to the respiratory mechanism, brings forth some very curious evidence.

As seen above, the large neotropical species of *Bufo* have developed relatively well-perfected lungs, concomitantly with a thick and glandular skin. The genus has, however, remained primitive as to life history. Even our largest species will spawn in any kind of standing water, including narrow and shallow ditches. The gelatinous ribbons of eggs extruded from the ovaries do not coalesce. The eggs spaced along them contain very inadequate yolk reserves. Hatching occurs in a very rudimentary condition, exposing the larvae to intense selection pressure, which must result in heavy mortality. The larval stage is shortened by early metamorphosis, at a very diminutive size, quite out of proportion to that reached by the adults, which include some of the largest neotropical anurans.

Eleutherodactylus, on the other hand, has scrapped the larval phase by developing directly in the egg, and has done away with the need for dual adaptation and remodelling organogeny. However, the adults of the species observed by us in southeastern Brazil have retained a very thin and moist skin. Their physiological requirements are so strict that they fail to survive when removed from their usual habitats.

All around biological improvement, opening up further possibilities, has not been achieved by representatives of either genus.

Evolutionary Significance

The modified anuran life histories already known, when placed in their proper sequence, form a number of linear series of ontogenetic evolution, leading from special aquatic to terrestrial environments and from abridged free swimming larval life to direct development. Some steps are missing but when the life histories of intermediate forms are discovered some of the gaps may be filled.

It is not altogether impossible that among the tropical and subtropical Salientia with unknown life histories there may be forms in which direct development has been achieved concomitantly with improvement of the lungs. But, for the time being, thought along these lines must remain speculative.

A number of neoevolutionists make a distinction between large scale and limited evolution. They might be inclined to

consider the caenogenetic changes in salientian development as mere specializations. And yet, the evolutionary trends of salientian ontogeny, modified towards: (1) better provision for the young; (2) withdrawal of development from selection pressure; (3) suppression of the dual adaptation to environment, foreshadow the conditions found in the more complex terrestrial classes of the vertebrate phylum.

The way of life of frogs with the usual moisture requirements is due less to excessive specialization than to an obligatory relegation to conditions which allow tetrapods with primitive lungs to survive in terrestrial environments. Idio-adaptation seems a better term to apply to them than specialization.

Evolution, or progress as he calls it, may come to an end, according to Huxley (1942), when all the possibilities inherent to a type of organism have been exploited. This is the case of the anurans under discussion. In such cases, at any rate, one cannot but ask, whether a basic, qualitative distinction between large scale and limited evolution really exists.

It seems legitimate to sum up by saying that the ancient Amphibia which were able to evolve further did not survive as modern relicts.

Acknowledgments

The author extends her best thanks to Drs. Charles M. Bogert, James A. Oliver and Ernst Mayr, for many helpful suggestions as to the presentation of the subject matter and for much trouble taken and interest shown in this paper; to Dr. Julian Huxley, for kindly reading and discussing an early draft with her, during his second visit to Brazil, and to Professor Costa Lima, for examining the table of insect enemies of tadpoles.

Summary

The ontogenetic evolution of frogs is discussed on the basis of a number of peculiar life histories observed in southeastern Brazil.

The general trend is towards withdrawal of development from water, with concomitant changes of reproductive behavior, ecotopic adaptations, progressive increase of yolk volume and acceleration of development.

Some of the changes are monophyletic, others recur in different families under similar ecological conditions. Spawning sites vary from special aquatic to manifold nonaquatic locations.

Withdrawal of development from water results in the reduction of selection pressure during ontogeny. This may have been an important factor in the clandestine evolution of the terrestrial vertebrates.

Progressive macrolecithism leads to lengthened intraoval life, with hatching as perfect, or as submetamorphic larva, or in adult shape. Increase of yolk is a first step towards improved provision for the young in oviparous vertebrates.

Morphological changes include differences in shape, the presence or absence of larval structures, and the reduction of nonfunctional organs. In macrolecithal eggs the embryos develop on top of the yolk. The limb buds of frogs with direct development form early, thus annulling one of the distinctions between the Amphibia and the Amniota.

Salientia with aquatic larvae and terrestrial adults require a dual adaptation to two successive environments. Sedentary, terrestrial tadpoles, nourished on yolk, and direct development do away with this need. They correspond to preliminary phases in the acceleration of development that has led to the disappearance of the larva and of metamorphosis from the life cycle of the higher vertebrates.

The failure of modern Salientia to evolve further is due to the dual respiratory mechanism, cutaneous and pulmonary, and to their primitive lungs. Some neotropical forms have achieved direct development, others have improved lungs. Forms may possibly yet be found in

which both improvements occur simultaneously.

The arrested evolution of the Salientia foreshadows the evolutionary achievements of the higher vertebrates. Though halted by the exhaustion of the biological possibilities inherent to their type of organism, no basic, qualitative distinction between their limited and large scale evolution is apparent.

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MORPHOLOGICAL DIFFERENCES AND PROBLEMS OF SPECIATION IN DROSOPHILA *

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Received August 25, 1947

Introduction

The usefulness of Drosophila in the development of genetic theory and as cytological material is known to all biologists, but it may not yet be obvious to all that the genus Drosophila offers highly favorable material also for the study of speciation. In the early days of Drosophila taxonomy separation of the species of the genus was accomplished by comparison of gross morphological traits. This method missed the interesting "borderline cases" between race and species but it was satisfying in that there was little difficulty involved in making out labels for individual dead specimens. If, however, the scientist becomes interested in the genetics, physiology, and environmental variations among the living members of the genus more and more refined methods are needed for the separation of the species within it. This is particularly true in Drosophila because here speciation is frequently accompanied by relatively little differentiation in morphological characters. The tendency to consider small differences, such as relative sizes of parts of the insect as useful indications of species differentiation, even to the point of separation of species on statistical differences, is no doubt exasperating to the museum man. Nevertheless, this is inevitable if we wish the species names to reflect the biological facts.

Data are presented in this article concerning small morphological differences between some of the "borderline cases" in the *obscura* group of Drosophila. They are of interest in that they show the kind and magnitude of some of the differences between groups of flies which have recently arrived at, or crossed, the threshold of species formation.

The authors wish to acknowledge the kindness of Professor Dobzhansky in furnishing all the stocks and many valuable suggestions.

Materials and Methods

Studies such as this one depend upon consistent methods and well controlled environmental conditions for meaningful answers. Large environmental fluctuations could cause greater variability in the quantitative characters studied than the genetic differences which interest us. About one-quarter of the data reported here were collected during 1941 and the rest during 1946–47. The same methods were used in both instances.

The flies which were measured were the offspring of single pair matings. Ten fertilized females of each geographic strain were placed singly in "creamers" and allowed to deposit eggs for two days. Each female was then moved to a fresh "creamer" and allowed to deposit eggs for another two days and so on. Fifty females and fifty males of each geographic strain which developed from these eggs were isolated upon emergence from the pupa cases and allowed to "harden" on fresh food in creamers for three days and then measured. The wings of the females and the wings and fore-legs of the males were mounted in clarite on glass slides, and coverslips applied. After drying, the

* Research accomplished at Harvard University and supported by a grant from the Milton Fund.

slides were projected on squared paper, a tracing made of the projection and the squares counted. The area of the wing in square millimeters was then obtained by conversion. The wing length was obtained by measuring the maximum length of the tracing on the squared paper followed by the application of a conversion factor. The teeth in the sex-combs were counted under the low power magnification (150 ×) of a compound microscope. These methods are those developed by the senior author for culturing and measuring flies to be used for flight studies as reported by Reed, Williams and Chadwick (1942).

All experimental animals were raised in a room having a constant temperature of 20.0 ± 0.5° C. Temperature control is of importance in all work concerned with wing size and the number of teeth in the sex-combs. Probably more important and more difficult to regulate is the quantity and quality of food material available to each larva.

Separation of Species in the Pseudoobscura Group by Means of Morphological Characters

One of the most interesting groups of "borderline species" known is composed of *D. miranda*, *D. pseudoobscura* (formerly Race A), *D. persimilis* (formerly Race B), and *D. subobscura*. All four species are very similar in most external characteristics. An attempt to classify individual dead specimens as to their species would be hazardous. Yet each of the four populations of flies is a true species according to the dynamic definition proposed by Dobzhansky which states that speciation has occurred when the stage of evolution has been reached "at which the once actually or potentially interbreeding array of forms becomes segregated in two or more separate arrays which are physiologically incapable of interbreeding." This definition may not have universal application but it does very well for the genus Drosophila.

There is extensive evidence all of which indicates that the first three of the above species do not form hybrids in nature even though their geographic ranges overlap in the western United States. The fourth species, *D. subobscura*, is European. A fifth species (or group of them), *D. obscura*, is also European and was not available for this study. Complete reproductive isolation between the four species seems to have been attained and gene interchange between them can be of little importance in nature. These considerations are in themselves enough to establish the four arrays of flies as distinct species according to the definition which we have accepted. Acceptance of this definition seems justified if systematic categories are to express the biological value of the populations concerned and not merely the degree of morphological distinctiveness.

We come now to the question of whether or not there are morphological differences between the four species which rest on differences in their genotypes.

Table 1. *Morphological differences between some of the species in the pseudoobscura group*

All flies grown at 20° C. Wing measurements from females only, while the counts of the sex-comb teeth are from their brothers. Standard errors are given for all measurements.

1. Species	2. No. of strains	3. Wing area in mm.²	4. Wing length in mm.	5. Reed's wing index no.	6. No. of teeth in ♂ sex-combs	
					Proximal	Distal
D. miranda	3	3.31 ± 0.02	3.31 ± 0.02	117.5	8.10 ± 0.08	5.92 ± 0.07
D. persimilis	10	2.75 ± 0.03	2.97 ± 0.01	71.9	6.30 ± 0.08	5.12 ± 0.09
D. pseudoobscura	14	2.50 ± 0.03	2.84 ± 0.02	57.3	6.90 ± 0.11	5.68 ± 0.08
D. subobscura	1	2.53 ± 0.03	2.83 ± 0.02	57.4	10.39 ± 0.24	10.00 ± 0.17

The answer is certainly in the affirmative. In order to find the answer the environmental conditions must be held quite constant for all the populations being compared, but that is not an unreasonable stipulation.

In table 1 may be found data on the morphological differences between the four species of the *D. pseudoobscura* group which are under consideration. The fifth column in the table gives what Dobzhansky and Epling (1944) have called "Reed's wing index." This index is calculated by multiplying the area of the wing in square millimeters by the length in millimeters cubed. This particular index was used because it had a relationship to the mechanics of flight as pointed out in Reed, Williams and Chadwick (1942) and gave values which were more useful than wing areas or lengths taken separately. A value combining both the area and the length of the wing adds significance beyond that provided by either measurement alone because two strains of flies may overlap in their wing area measurements but not in their measurements of wing length, or vice versa. If wing area and wing length were perfectly correlated, the index would be useless, but as they are not perfectly correlated, the index provides something in addition to the magnification of the small differences.

Further significance is added to the distinction between *D. miranda* and the other three species by observing the count of the teeth in the sex-combs of the males. Following a consideration of the wing index and the number of teeth in the sex-combs there should be no difficulty in classifying the progeny of individual females captured in nature as to whether or not the strain is *D. miranda*.

The problem of distinguishing *D. subobscura* from the other three species is of academic interest only, because there is no overlapping of its European range with that of the three American species. There is no significant difference between the wing index of *D. pseudoobscura* and *D. subobscura* as may be seen in table 1. The large number of teeth in the sex-combs of *D. subobscura* are a morphological character which in itself would be sufficient to determine whether the progeny of a female belong to that species or not.

It is established that if the flies are raised under uniform conditions, progeny of *D. miranda* and *D. subobscura* can be distinguished from *D. pseudoobscura* and *D. persimilis* progeny on purely morphological characters. Can these characters be used to distinguish the more closely allied pair of species, *D. pseudoobscura* and *D. persimilis* from each other? Table 1 shows that while the differences in wing measurements and sex-comb teeth counts are small, they are, none-the-less, highly significant. For example, the difference between the wing areas of *D. persimilis* and *D. pseudoobscura* is only 0.25 ± 0.042 square millimeters, but this difference is 5.9 times its standard error, and is therefore highly significant.

Finally, then, we may say that all four species considered here can be separated on purely morphological characters provided an adequate number of flies, all of which have been raised under the same conditions, are examined. The differences between the four species are highly significant statistically though not of great magnitude. They are differences in degree which are real and rest upon a genetic basis.

The Consistency of the Species Differences

Let us examine the consistency of the results on wing area and wing length obtained by Reed, Williams and Chadwick (1942) and the more recent data obtained by us. The average area of the wings of five strains of *D. pseudoobscura* as shown in the earlier paper was 2.44 square millimeters compared with an average of 2.53 square millimeters for nine recently measured strains of the same species. For *D. persimilis* the areas were 2.73 and 2.76 square millimeters respec-

tively. The wing lengths for *D. pseudoobscura* were 2.83 millimeters in the earlier paper and 2.84 in this one. Finally, wing lengths of *D. persimilis* were 2.98 millimeters and 2.96 millimeters respectively.

The above differences are small and except for the difference in areas between the five strains of *D. pseudoobscura* given in the first study and the nine in this study there is no suspicion of any true difference, either genetic or environmental, between the results recorded in the first paper and those just completed. The difference in wing area of nine hundredths of a square millimeter between the two samples of *D. pseudoobscura* is statistically significant but not of sufficient magnitude to affect the general finding of a highly significant difference in wing area between *D. pseudoobscura* and *D. persimilis*. Considering the fact that none of the five strains making up the average in the first study was the same as any one of the nine strains used in the present study, it is clear that our results obtained during the two studies are reliable and can be reproduced under our experimental conditions.

Let us see how our results compare with those of Mather and Dobzhansky (1939) which were obtained under remarkably different environmental conditions. They had no wing measurements that are strictly comparable to ours but provided abundant counts of the teeth in the sex-combs. The culture technique of Mather and Dobzhansky was quite different from ours in that as many as 20 females were allowed to oviposit in a half-pint milk bottle while we used a single female in each creamer. Consequently each larva in our experiment might be expected to have more available area of food surface and to produce a larger fly with more teeth in the sex-comb. This seems to have been the case as our results, shown in tables 2 and 3, are a little higher in frequency of teeth than Mather and Dobzhansky might have obtained. Bearing this in mind, our results fit into the picture very well indeed and give additional support to their finding that lowering the temperature in-

TABLE 2. *Frequencies of teeth in the proximal and distal sex-combs of* D. pseudoobscura *at different temperatures and in different laboratories*

Author	No. of strains	Temperature	No. of teeth in sex-comb	
			Proximal	Distal
Mather and Dobzhansky ('39)	4	17.5° C.	6.95 ± 0.17	5.57 ± 0.15
Reed and Reed (table 4 of this paper)	14	20.0° C.	6.90 ± 0.11	5.68 ± 0.08
Mather and Dobzhansky ('39)	11	24.5° C.	6.46 ± 0.09	5.32 ± 0.07
Dobzhansky ('35)	5	24.5° C.	6.57 ± 0.05	5.22 ± 0.05

TABLE 3. *Frequencies of teeth in the proximal and distal sex-combs of* D. persimilis *at different temperatures and in different laboratories*

Author	No. of strains	Temperature	No. of teeth in sex-comb	
			Proximal	Distal
Mather and Dobzhansky ('39)	4	17.5° C.	5.81 ± 0.16	5.16 ± 0.13
Reed and Reed (table 5 of this paper)	10	20.0° C.	6.30 ± 0.08	5.12 ± 0.09
Mather and Dobzhansky ('39)	12	24.5° C.	5.55 ± 0.12	4.73 ± 0.12
Dobzhansky ('35)	3	24.5° C.	5.77 ± 0.06	4.81 ± 0.06

creases the number of teeth in both the proximal and distal combs.

The important observation which comes from a comparison of the data in tables 2 and 3 is that even with different culture methods and over a wide range of temperatures no value for the teeth in *D. persimilis* ever gets as high as the lowest value for *D. pseudoobscura* even when as few as three geographic strains (total, 88 flies) were used. Consequently, if about five strains are used, a clear cut difference between the two species would be expected under any likely culture conditions and temperature. There is little doubt then, that the differences between the species in regard to the frequency of teeth in the sex-combs are genetic.

Morphological Differences Between Geographic Strains of D. Persimilis and D. Pseudoobscura

There is no reasonable doubt about the significance of the differences between *D. pseudoobscura* and *D. persimilis* as shown in tables 1, 2, and 3. The averages shown there are based upon fourteen and ten different strains respectively and therefore have considerable stability. The differences between the averages based on *several* strains are highly significant but it is not true that the difference in a single characteristic between a particular geographic strain of *D. pseudoobscura* and one of *D. persimilis* is always significant. Occasionally a strain of *D. pseudoobscura* will be as large as a small strain of *D. persimilis*. It should be borne in mind that each of our geographic strains is composed of the descendants of a single fertilized female captured in nature. If this female deviated greatly from the mean for her species in size, genetically, it is quite possible for her offspring of later generations which were measured in the laboratory to do likewise. However, this female is not as likely to "overlap" with the other species in characters which are imperfectly correlated with the first character. It will be seen in table 4 that four out of fourteen strains of *D. pseudoobscura* have wings with areas as those of the two smallest strains of *D. persimilis*. With wing length, however, only one of the strains of *D. pseudoobscura* overlaps with *D. persimilis*. As wing area and wing length are not perfectly correlated, "Reed's wing index" becomes useful and it can be seen from table 4 that none of the strains of the two species give index numbers which overlap. If the frequency of the teeth in the sex-combs be considered, there is again overlapping between strains of the two species which is somewhat worse than it was with wing area and considerably worse than with wing length. However, as teeth in the sex-combs are imperfectly correlated with the wing measurements, it is possible to construct an index combining wing measurements and frequency of teeth in the sex-combs. As it is of no theoretical importance which wing measurement is used, the less advantageous one for our argument was chosen; that is wing area. Wing area was multiplied by 100 in order to dispense with two decimal places and then divided by the product of the number of teeth in the proximal comb times the number of teeth in the distal comb. This "new index" is given as the last column of tables 4 and 5. It can be seen that there is no overlapping of the "new index" numbers for the two species. The "new index" is somewhat better than "Reed's wing index" due to the fact that sex-combs and wing areas are not as highly correlated as wing areas and wing lengths.

The morphological data for males are given in table 5. Except for the smaller dimensions they show no fundamental differences from those of the females. Wing sizes of males of *D. persimilis* are larger than those of *D. pseudoobscura* as one would expect from the data for the females. Mather and Dobkhansky (1939) found the wing dimensions of *D. persimilis* females to be greater than those of *D. pseudoobscura*. They found the reverse to be true for males, however, which is not a reasonable answer in the light

of our present knowledge. All the evidence from developmental rates, type of habitate, etc., indicates that *D. persimilis* should have the larger wings of the two species and our results show that this is certainly true for males, as well as for females for which there is no conflicting evidence. It is possible that the males behaved differently from the females in the experiments of Mather and Dobzhansky (1939) at 24.5° C. and under their particular environmental conditions, but it is more likely that their results were due to statistical fluctuations in the case of the males, or other causes which are not known to us.

It is clear that the variation between geographic strains is so great that classification of a strain on the basis of *one* character only, say wing area, is dangerous and not to be recommended. The use of an index number involving two or more characters therefore becomes profitable. Were it not for the fortuitous advantage offered by the salivary gland chromosomes, the use of some such index num-

TABLE 4. *Morphological differences between* females *of* D. pseudoobscura *and* D. persimilis. *Frequencies of the teeth in the sex-combs obtained from their brothers. Standard errors are given for all measurements*

Strain ♀ ♀	Wing area in mm.²	Wing length in mm.	Reed's wing index	No. of teeth in ♂ sex-combs		New index
				Proximal	Distal	
Pseudoobscura						
Willapa Bay*	2.31 ± 0.01	2.83 ± 0.01	52.72	6.11 ± 0.08	5.18 ± 0.08	7.3
Perpetua*	2.23 ± 0.02	2.73 ± 0.02	45.72	6.95 ± 0.09	5.87 ± 0.06	5.5
Kaibab*	2.48 ± 0.04	2.84 ± 0.02	56.80	6.72 ± 0.08	5.72 ± 0.07	6.5
Prescott*	2.53 ± 0.03	2.88 ± 0.02	60.44	6.51 ± 0.08	5.54 ± 0.07	7.0
Quezaltenango*	2.63 ± 0.03	2.88 ± 0.02	62.84	6.56 ± 0.07	5.31 ± 0.07	7.6
Piñon Flats 140	2.47 ± 0.01	2.81 ± 0.02	54.81	6.84 ± 0.05	6.10 ± 0.06	5.9
Piñon Flats 6	2.61 ± 0.02	2.94 ± 0.01	66.32	7.31 ± 0.07	5.85 ± 0.07	6.1
Piñon Flats 11	2.42 ± 0.02	2.80 ± 0.01	53.12	6.57 ± 0.08	5.13 ± 0.05	7.2
Piñon Flats 103	2.54 ± 0.01	2.85 ± 0.01	58.80	6.75 ± 0.08	5.66 ± 0.06	6.6
Tuolumne Meadow	2.50 ± 0.01	2.85 ± 0.01	57.88	7.01 ± 0.07	5.62 ± 0.06	6.3
Aspen Valley	2.52 ± 0.02	2.87 ± 0.02	59.57	7.19 ± 0.07	6.07 ± 0.06	5.8
Mather	2.46 ± 0.02	2.73 ± 0.01	50.06	7.77 ± 0.08	5.95 ± 0.05	5.3
Lost Claim	2.67 ± 0.03	2.88 ± 0.02	63.79	7.34 ± 0.09	5.60 ± 0.08	6.5
Jacksonville	2.60 ± 0.02	2.85 ± 0.01	60.19	6.90 ± 0.08	5.92 ± 0.06	6.4
Average	2.50 ± 0.03	2.84 ± 0.02	57.36	6.90 ± 0.11	5.68 ± 0.08	6.4
Range	2.23–2.67	2.73–2.94	45.72–66.32	6.11–7.77	5.13–6.10	5.3–7.6
Persimilis						
Willapa Bay*	2.74 ± 0.02	3.00 ± 0.01	74.00	6.27 ± 0.08	5.63 ± 0.08	7.8
Perpetua*	2.82 ± 0.03	3.00 ± 0.02	76.16	6.58 ± 0.08	5.32 ± 0.05	8.1
Stony Creek*	2.60 ± 0.02	2.98 ± 0.02	71.32	6.20 ± 0.10	4.80 ± 0.09	8.7
Yolla Bolly*	2.75 ± 0.03	2.96 ± 0.02	68.80	5.89 ± 0.08	4.64 ± 0.07	10.1
Hope	2.62 ± 0.03	2.96 ± 0.01	67.94	6.28 ± 0.07	5.00 ± 0.06	8.3
Sequoia	2.78 ± 0.01	2.92 ± 0.01	69.22	6.38 ± 0.06	5.22 ± 0.07	8.3
Tuolumne Meadow	2.97 ± 0.02	2.96 ± 0.01	77.01	6.67 ± 0.07	5.27 ± 0.06	8.4
Porcupine Flat	2.78 ± 0.02	2.94 ± 0.01	70.64	5.91 ± 0.07	5.02 ± 0.05	9.4
Aspen Valley	2.76 ± 0.02	3.04 ± 0.01	77.52	6.20 ± 0.06	5.11 ± 0.05	8.7
Lost Claim	2.68 ± 0.02	2.92 ± 0.01	66.73	6.60 ± 0.08	5.17 ± 0.05	7.9
Average	2.75 ± 0.03	2.97 ± 0.01	71.93	6.30 ± 0.08	5.12 ± 0.09	8.6
Range	2.60–2.97	2.92–3.00	66.73–77.52	5.91–6.60	4.64–5.63	7.8–10.1

* Data for these strains obtained in 1941. Data for the others obtained in 1946–47.

TABLE 5. *Morphological differences between* males *of* D. pseudoobscura *and* D. persimilis. *Standard errors are given for all measurements*

Strain ♂♂	Wing area in mm.²	Wing length in mm.	Reed's wing index	No. of teeth in sex-combs		New index
				Proximal	Distal	
Pseudoobscura						
Piñon Flats 140	2.12 ± 0.01	2.69 ± 0.01	41.27	6.84 ± 0.05	6.10 ± 0.06	5.1
Piñon Flats 6	2.19 ± 0.01	2.67 ± 0.01	41.67	7.31 ± 0.07	5.85 ± 0.07	5.1
Piñon Flats 11	2.06 ± 0.01	2.62 ± 0.01	37.04	6.57 ± 0.08	5.13 ± 0.05	6.1
Piñon Flats 103	2.06 ± 0.01	2.62 ± 0.01	37.04	6.75 ± 0.08	5.66 ± 0.06	5.4
Tuolumne Meadow	2.02 ± 0.01	2.56 ± 0.01	33.90	7.01 ± 0.07	5.62 ± 0.06	5.1
Aspen Valley	2.12 ± 0.01	2.58 ± 0.01	36.40	7.19 ± 0.07	6.07 ± 0.06	4.9
Mather	2.06 ± 0.02	2.50 ± 0.01	32.20	7.77 ± 0.08	5.95 ± 0.05	4.5
Lost Claim	2.26 ± 0.02	2.67 ± 0.01	43.01	7.34 ± 0.09	5.60 ± 0.08	5.5
Jacksonville	2.15 ± 0.01	2.56 ± 0.01	36.08	6.90 ± 0.08	5.92 ± 0.06	5.3
Average	2.12 ± 0.02	2.61 ± 0.02	37.62	7.08 ± 0.12	5.77 ± 0.10	5.2
Range	2.02–2.26	2.50–2.69	32.30–43.01	6.57–7.77	5.13–6.10	4.5–6.1
Persimilis						
Hope	2.24 ± 0.01	2.75 ± 0.01	46.59	6.28 ± 0.07	5.00 ± 0.06	7.1
Sequoia	2.23 ± 0.01	2.70 ± 0.01	43.89	6.38 ± 0.06	5.22 ± 0.07	6.7
Tuolumne Meadow	2.51 ± 0.01	2.85 ± 0.01	58.11	6.67 ± 0.07	5.27 ± 0.06	7.1
Porcupine Flat	2.31 ± 0.02	2.75 ± 0.01	48.05	5.91 ± 0.07	5.02 ± 0.05	7.9
Aspen Valley	2.19 ± 0.02	2.69 ± 0.02	42.64	6.20 ± 0.06	5.11 ± 0.05	6.9
Lost Claim	2.24 ± 0.02	2.69 ± 0.01	43.61	6.60 ± 0.08	5.17 ± 0.05	6.6
Average	2.29 ± 0.05	2.74 ± 0.02	47.15	6.34 ± 0.11	5.13 ± 0.04	7.0
Range	2.19–2.51	2.69–2.85	42.64–58.11	5.91–6.67	5.00–5.27	6.6–7.9

ber or similar method for classification of the strains as to their species would be the only morphological method available.

Similar systems of index numbers have been used on various occasions, one of the most interesting studies having been made by Anderson and Whitaker (1934) on the separation of *Uvularia grandiflora* from *Uvularia perfoliata*.

THE CONSISTENCY OF THE STRAIN DIFFERENCES

One of the remarkable aspects of tables 4 and 5 is the uniformity within the samples of animals measured. It will be noticed, for example, that all the standard errors are very small. Consequently the dimensions of many of the "geographic strains" are statistically significantly different from some of the other geographic strains of their own species.

The small standard errors obtained for the samples of single strains indicate that there is little genetic segregation or individual variability within a sample of a strain. It is quite possible, however, that samples studied at different times, even with our fairly constant culture technique, might vary significantly. Such was the case. Table 6 provides a good picture of the variability to be found in one strain. The character studied was the number of teeth in the sex-combs and it can be seen that there was considerable fluctuation in the counts obtained. The maximum difference in counts was five or six times its standard error and was consequently highly significant. The main diference to be observed is the one related to time. The counts in 1941 were lower than those taken in 1946–47. This is the largest and most important varia-

TABLE 6. *Comparison of effects of environmental fluctuations, statistical chance factors, and personal equations on the number of teeth in the sex-combs of a strain of* D. persimilis. *All at 20° C.*

Strain	Sample no.	No. of feet in sample	Date prepared	Av. no. of teeth		Investigator
				Proximal	Distal	
Yolla Bolly	1	55	July '41	5.89 ± 0.08	4.64 ± 0.04	S. C. Reed
Yolla Bolly	2	43	Nov. '41	5.74	4.54	E. Hunt
Yolla Bolly	3	100	Oct. '46	5.96 ± 0.06	4.84 ± 0.04	E. W. Reed
Yolla Bolly	4	100	June '47	6.31 ± 0.05	5.06 ± 0.04	E. W. Reed
Yolla Bolly	4	100		6.27	5.03	S. C. Reed (Recount of June '47 slides)

tion demonstrated by the strain. It is not restricted to this strain, however, as is indicated by an average count of 6.57 teeth in the proximal comb of five strains of *D. pseudoobscura* done in 1941, compared with a count of 7.08 teeth for nine strains of the same species done in 1946–47. The 1941 counts were done by S. C. Reed and the 1946–47 counts by E. W. Reed. A recount by S. C. Reed of slides counted by E. W. Reed shows no significant deviation, as demonstrated in the last two lines of table 6. The personal equation seems to be of no importance. It is doubtful whether the genotype has changed consistently between 1941 and 1946 in these strains, so we are left with the conclusion that the major fluctuations in our data result from uncontrolled changes in the culture technique. The fluctuation in the number of teeth in the sex-combs seems to have been proportionally much greater than in the wing areas and lengths following the change in conditions. We have no knowledge as to what feature of our technique has varied though it seems most likely that there has been some change in the composition or consistency of the food itself.

SUMMARY AND CONCLUSIONS

The four species *D. miranda, D. persimilis, D. pseudoobscura* and *D. subobscura* are true species because their reproductive isolation seems to have become irrevocable. They are excellent illustrations of a characteristic of the genus Drosophila in which speciation is frequently accompanied by relatively little differentiation in morphological characters. Apparently these four species are adapted to their ecological niches with such precision that genotypes causing any extensive changes in morphology would be eliminated by natural selection. Most genes causing large morphological changes apparently have a poor chance of survival, but those causing small changes in morphology may be favored because they would allow the group of closely related species to utilize the ecological range more completely than a single species could.

The three American species show a gradation in wing size which is correlated with climate. The species living in the coldest environment, *D. miranda,* has the largest wings. *D. pseudoobscura* has the smallest wings and inhabits the warmest regions. *D. persimilis* is intermediate, though closer to *D. pseudoobscura,* in both respects. The genotypes of all three species are fluid and individuals which deviate greatly from the mean of their own species in regard to any one characteristic could be misclassified if only one morphological character were considered. If an adequate sample of a species is measured under uniform conditions, there will be little doubt as to which species it is. If more than one morphological character is considered and some sort of index number calculated, there will be much less doubt as to which of the species the array belongs. In this paper two characters were

employed in the study of morphological differences between the species. These were wing size and the number of teeth in the sex-combs. With only two morphological characters it was possible to classify each strain as to its species. The absolute differences were small, as expected, but significant. The calculation of an index number including other morphological characteristics in addition to the ones considered here would give even sharper differentiation and would no doubt permit classification of specimens, as well as populations.

The results of this paper show that the four forms of Drosophila, (*D. miranda, D. persimilis, D. pseudoobscura,* and *D. subobscura*), are not only true species on the grounds of reproductive isolation, but that they are genetically different in regard to the morphological characters studied. Therefore, they differ from the so-called "good" morphological species only in degree, that is, in the magnitude of the morphological differences.

It is clear that the difficulties inherent in a system of classification based entirely upon such small morphological differences may be considerable. Consequently the chances of devising some system of classification for the genus Drosophila which will be wholly satisfactory to the museum man are small, if the system is to be based upon genetic divergence protected by reproductive isolation. However, the only system that will give an accurate picture of the evolutionary status of closely related groups of the genus Drosophila will be one which is based upon a decision as to whether gene exchange between the groups is proceeding at a faster rate than gene divergence within the groups, or whether it is likely to do so in the future.

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MICROGEOGRAPHIC ANALYSIS OF CHROMOSOMAL VARIATION IN A CHINESE SPECIES OF *CHIRONOMUS* (DIPTERA)

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Received October 15, 1947

Introduction

Prominent among the problems in the field of population genetics is the inquiry into the causal factors of changes in the genetic structure of free-living populations. In recent years, a considerable amount of information has been gained through the analysis of chromosomal variation of dipteran insects, particularly the work of Dobzhansky and his associates on *Drosophila pseudoobscura* and *Drosophila persimilis* (see reviews by Dobzhansky, 1941; White, 1945). This has been made possible largely because of the development of the salivary gland chromosome technique which permits direct observation of the detailed structure of the chromosomes, thus revealing the distinct characteristics of various kinds of chromosomal structural hybridity.

Another dipteran insect, the midge, *Chironomus*, although it is difficult to breed, offers one advantage over *Drosophila* for population studies on chromosomal variation, that is, the analysis can be done by examining the salivary gland chromosomes of the larvae collected directly in [illegible]. It was Bauer (1935) who first demonstrated the presence of inversions as well as intrachromosomal translocations in several species of *Chironomus*. More recently, Philip (1942) noticed the following species differences. While most populations of *Chironomus riparius* and *C. dorsalis* contain one or more inversions, the material of *C. thummi* from six different localities in England and Germany did not show a single inversion. She showed further that the various gene arrangements are combined at random, and that the differences between different geographical populations in the frequency of some arrangements are not significant.

In this paper we are going to present the results of an analysis of six different chromosomal variants found in *Chironomus* species[1] inhabiting three semi-isolated localities. While we agree with both Bauer and Philip that the different variants combine at random, it is, however, clear to us that there do exist real differences in the frequencies of some gene arrangements among the three different populations.

Materials and Methods

Larvae of *Chironomus* species were collected from small muddy ponds of three different localities in the vicinity of Meitan, Kweichow Province, China. These localities are Tamiochang, Tangchiatze, and Matisze. These three places lie in a nearly straight line, with Tamiochang and Matisze at the two ends and Tangchiatze in between, the approximate distance between Tamiochang and Tangchiatze being 7.5 kilometers and that between Tangchiatze and Matisze being 3.5 kilometers. Breeding places of *Chironomus* were found more abundant in Tamiochang and Tangchiatze as compared to Matisze; between the localities some small breeding pools may also be noticed, especially soon after rainfall. For our pur-

[1] Larval material of the species was submitted by the authors but it was impossible to have it identified.—Ed.

pose, larvae were collected only from pools that contained fairly large populations, numbering more than one thousand.

All collections were made during the two months, June and July of 1945. In the late summer, severe drought dried up these pools and probably killed most of the remaining larvae.

Individual salivary gland chromosome preparations were made from full grown larvae previous to the stage of thoracic swelling. The ordinary aceto-carmine smear method was used with one modification, that is, the gland was first treated with normal hydrochloric acid at room temperature for about five minutes before being dipped into aceto-carmine solution. This pretreatment with acid hydrolysis, according to Chu (1946), enables one to remove any stainable matter in the cytoplasm, thus rendering a more distinct differentiation of the chromosomes. Permanent preparations were made with the aid of euparal mounting.

Types of Chromosome Variations

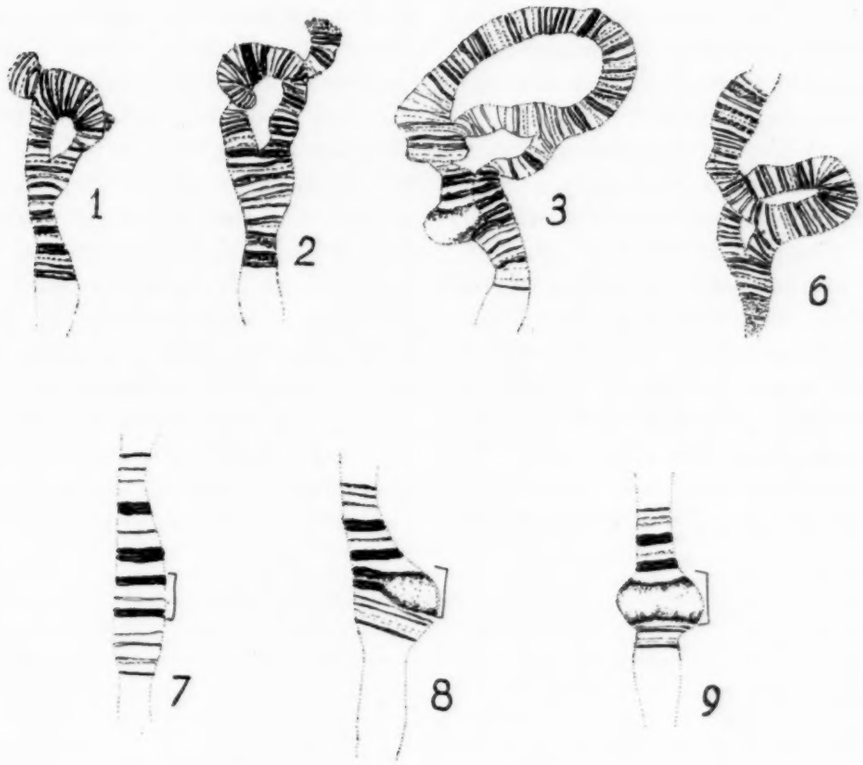
Like the other *Chironomus* species described in the literature (Bauer, 1935; Philip, 1942; Goldschmidt, 1942), our species has four pairs of chromosomes. In the salivary gland nuclei there is no chromocenter connecting the centromere regions, and the individual chromosomes can be easily traced, as they are rather widely separated from each other. The four chromosomes can be readily recognized by their relative lengths as well as by the characteristic features of their respective free ends. They are numbered from 1 to 4 in the order of their lengths, the first chromosome being the longest and the fourth the shortest in the salivary gland nucleus.

There were identified a total of six different chromosome rearrangements of which five are paracentric inversions and the sixth is a bulb-like modified structure similar to that described by Goldschmidt (1942) in a Palestinian *Chironomus* species. Two of the inversions known as 1_a and 1_b (figs. 1 and 2) are located in the two separate arms of the first chromosome, having one break in each case lying very close to the free end. In the second chromosome, there were also observed two different inversions. One of them (2_a) is a simple inversion having one break apparently near the free end (fig. 3), and the other (2_b), which is found on the other arm of the chromosome, presents a complicated configuration in the salivary gland nuclei of the heterozygotes. In favorable preparations, the heterozygotes show a succession of three loops. This was later identified to be a tandem inversion involving four breaks, the last one lying very close to the end. Furthermore, one of the two gene arrangements seems to be devoid of terminal heterochromatin. The loss might have occurred during the process of inversion (figs. 4 and 5). The fifth inversion, to be known as 3_a, which is found on the third chromosome, is again a simple subterminal one (fig. 6).

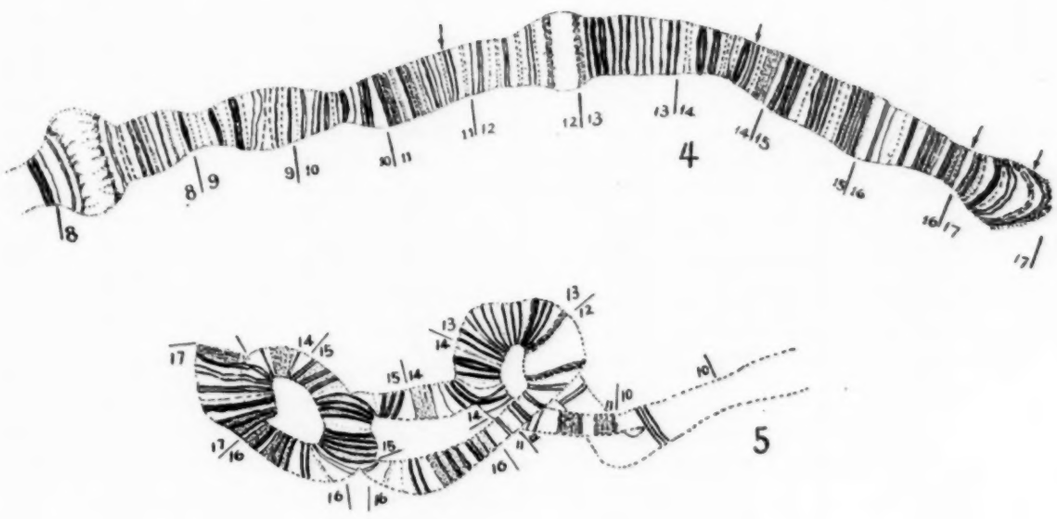
Of particular interest is the bulb-like structure. As shown in figure 3, it is located not far from the proximal end of the inversion 2_a on the second chromosome. In the heterozygote this gives the appearance of a conspicuous swollen structure corresponding to a region of four heavy and one light bands in the normal chromosome. Figures 7 to 9 show the said region from a normal homozygote, a "bulb" heterozygote, and a "bulb" homozygote respectively. Because of the distinctness with which the three classes can be distinguished, the frequency distribution data include the heterozygotes and the two classes of homozygotes as well. In fact, this variation constitutes the only type reported in this paper that gives complete information about the relative frequencies of the heterozygotes and the homozygotes.

Analysis

Excepting the case of 2_c, the data available for five inversions involve the heterozygotes only. Table 1 presents the



FIGS. 1–9, excluding 4–5



FIGS. 4–5

Fig. 1. Inversion 1_a; fig. 2. Inversion 1_b; fig. 3. Inversion 2_a with heterozygous bulb; fig. 4. Map of a portion of the cromosome 2, arrows indicating the breakage points of the inversion 2_b; fig. 5. Inversion 2_b; fig. 6. Inversion 3_a; fig. 7. Homozygous non-bulb; fig. 8 Heterozygous bulbs; fig. 9. Homozygous bulb.

summary of the analysis of the three populations. The expected frequencies are calculated according to the assumption of independent combination and random mating. In each class a separate χ^2 value is given for the purpose of testing the significance of the difference. Since each inversion was recorded by its heterozygous representatives only, the frequency of homozygotes is limited by the total number, thus reducing one degree of freedom in each case. For six different variable types as listed in table 1, the total number of classes being sixty-four, we can have only fifty-seven degrees of freedom for each population. With few exceptions, the distribution and combination of the different variants in each population fit well the expected frequencies. Even in cases involving the combinations of the variants on the same chromosome, our data do not seem to indicate any significant linkage. The exceptional cases which show very high χ^2 values are the following combinations: $1_a1_b2_b$ and $1_a2_a2_c$ of Tamiochang, 2_a and $1_a2_b2_c3_a$ of Tangchiatze, and $1_a1_b2_b$ of Matisze. Nevertheless, the probability range of the total χ^2 values is still in accord with the assumption of random mating. It is probable that the few cases showing statistically significant differences may be due to sampling errors.

Whether or not these chromosomal

TABLE 1. *Inversion contents in the three populations*

Inversion combinations	Tamiochang			Tangchiatze			Matisze		
	obs.	exp.	χ^2	obs.	exp.	χ^2	obs.	exp.	χ^2
0	32	24.05	2.63	20	21.22	0.07	45	39.54	0.75
1a	0	2.51	2.51	4	4.37	0.03	3	3.66	0.12
1b	3	4.92	0.75	6	8.44	0.70	2	4.48	1.16
2a	3	1.96	0.55	13	5.69	9.41	2	3.26	0.49
2b	4	7.82	1.86	7	7.78	0.08	6	9.57	1.33
2c	17	18.44	0.11	12	14.05	0.30	27	27.11	0.00
3a	5	7.42	0.79	3	4.12	0.30	2	3.26	0.49
1a1b	0	0.51	0.51	2	1.74	0.04	0	0.41	0.41
1a2a	0	0.21	0.21	1	1.17	0.03	0	0.30	0.30
1a2b	1	0.81	0.04	2	1.60	0.10	1	0.89	0.02
1a2c	2	1.92	0.00	5	2.89	1.54	2	2.51	0.10
1a3a	0	0.77	0.77	0	0.85	0.85	1	0.30	1.61
1b2a	0	0.40	0.40	3	2.26	0.24	0	0.37	0.37
1b2b	2	1.60	0.10	2	3.09	0.39	3	1.08	3.39
1b2c	1	3.77	2.04	4	5.59	0.45	3	3.07	0.00
1b3a	3	1.52	1.44	5	1.64	6.90	1	0.37	1.07
2a2b	0	0.64	0.64	0	2.09	2.09	0	0.79	0.79
2a2c	2	1.51	0.16	6	3.77	1.33	5	2.24	3.41
2a3a	0	0.61	0.61	0	1.10	1.10	0	0.27	0.27
2b2c	7	5.99	0.17	7	5.15	0.66	4	6.56	1.00
2b3a	3	2.41	0.14	1	1.51	0.17	2	0.79	1.85
2c3a	7	5.69	0.30	1	2.73	1.10	2	2.24	0.03
1a1b2a	0	0.04	0.04	0	0.47	0.47	0	0.03	0.03
1a1b2b	2	0.17	20.12	1	0.64	0.21	2	0.10	36.10
1a1b2c	1	0.39	0.94	0	1.15	1.15	0	0.28	0.28
1a1b3a	0	0.16	0.16	1	0.34	1.30	0	0.03	0.03
1a2a2b	0	0.07	0.07	0	0.43	0.43	0	0.07	0.07
1a2a2c	2	0.16	21.64	2	0.78	1.94	0	0.21	0.21
1a2a3a	0	0.06	0.06	0	0.23	0.23	0	0.03	0.03
1a2b2c	0	0.62	0.62	1	1.06	0.00	1	0.61	0.25
1a2b3a	0	0.25	0.25	1	0.31	1.53	0	0.07	0.07
1a2c3a	0	0.59	0.59	0	0.56	0.56	0	0.21	0.21
1b2a2b	0	0.13	0.13	0	0.83	0.83	0	0.09	0.09

TABLE 1—*Continued*

Inversion combinations	Tamiochang			Tangchiatze			Matisze		
	obs.	exp.	χ^2	obs.	exp.	χ^2	obs.	exp.	χ^2
1b2a2c	0	0.31	0.31	0	1.50	1.50	0	0.25	0.25
1b2a3a	0	0.12	0.12	0	0.44	0.44	0	0.03	0.03
1b2b2c	1	1.23	0.04	4	2.05	1.86	1	0.74	0.09
1b2b3a	2	0.49	4.59	2	0.60	3.26	0	0.09	0.09
1b2c3a	1	1.16	0.02	1	1.09	0.01	0	0.25	0.25
2a2b2c	0	0.49	0.49	1	1.38	0.11	2	0.54	3.92
2a2b3a	0	0.20	0.20	0	0.41	0.41	0	0.07	0.07
2a2c3a	1	0.47	0.62	0	0.73	0.73	0	0.21	0.21
2b2c3a	1	1.85	0.39	1	1.00	0.00	1	0.54	0.39
1a1b2a2b	0	0.01	0.01	0	0.17	0.17	0	0.01	0.01
1a1b2a2c	0	0.03	0.03	0	0.31	0.31	0	0.02	0.02
1a1b2a3a	0	0.01	0.01	0	0.09	0.09	0	0.00	0.00
1a1b2b2c	1	0.13	5.94	0	0.42	0.42	0	0.05	0.05
1a1b2b3a	0	0.05	0.05	0	0.12	0.12	0	0.01	0.01
1a1b2c3a	0	0.12	0.12	1	0.22	2.71	0	0.02	0.02
1a2a2b2c	0	0.05	0.05	0	0.28	0.28	0	0.05	0.05
1a2a2b3a	0	0.05	0.05	0	0.08	0.08	0	0.01	0.01
1a2a2c3a	0	0.05	0.05	0	0.15	0.15	0	0.02	0.02
1a2b2c3a	1	0.19	3.37	0	0.21	0.21	0	0.05	0.05
1b2a2b2c	0	0.10	0.10	0	0.55	0.55	0	0.06	0.06
1b2a2b3a	0	0.04	0.04	0	0.16	0.16	0	0.01	0.01
1b2a2c3a	0	0.10	0.10	0	0.29	0.29	0	0.02	0.02
1b2b2c3a	1	0.38	1.02	3	0.40	17.01	0	0.06	0.06
2a2b2c3a	0	0.15	0.15	0	0.27	0.27	0	0.05	0.05
1a1b2a2b2c	0	0.01	0.01	0	0.11	0.11	0	0.01	0.01
1a1b2a2b3a	0	0.00	0.00	0	0.03	0.03	0	0.00	0.00
1a1b2a2c3a	0	0.01	0.01	0	0.06	0.06	0	0.00	0.00
1a1b2b2c3a	0	0.04	0.04	0	0.08	0.08	0	0.01	0.01
1a2a2b2c3a	0	0.02	0.02	0	0.06	0.06	0	0.00	0.00
1b2a2b2c3a	0	0.03	0.03	0	0.11	0.11	0	0.01	0.01
1a1b2a2b2c3a	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
Total	106		79.33	123		68.12	118		62.10
P		0.05			0.29–0.30			0.60–0.61	

variations existing in nature are in an equilibrium state cannot be answered from the data on the five inversions, where, in each case, the frequencies of individuals homozygous for the normal and for the inverted sequence are combined. It is only the variant 2_c, for which the frequencies of both homozygotes and of heterozygotes are given, that permits a consideration of the question of equilibrium.

The frequencies of gene arrangements can be calculated as proposed by Haldane (1938). Let the frequency of the homozygous bulb be represented by "a," of the heterozygous bulb by "b," and of the homozygous non-bulb by "c." On the assumption of random mating and equal viability, the expected frequencies of the two sequences should be

$$\text{bulb} \quad 2a + b \qquad \text{non-bulb} \quad b + 2c.$$

If mating is at random in a sample consisting of n zygotes we will expect:

$$\text{bulb} \quad 2np \qquad \text{non-bulb} \quad 2n(1 - p)$$

where p and $(1 - p)$ represent the theoretical frequencies of bulb and non-bulb arrangements respectively. The logarithm of likelihood of the observation is

$$L = (2a + b) \log p + (b + 2c) \log (1 - p).$$

Hence,

$$\frac{dL}{dp} = \frac{2a + b}{p} - \frac{b + 2c}{1 - p} = 0$$

and

$$p = \frac{2a + b}{2n}.$$

The standard deviation for p and $(1 - p)$ is

$$\pm \sqrt{\frac{(2a + b)(b + 2c)}{8n^3}}.$$

Table 2 shows the proportions of the two sequences in the three populations. On the basis of the values p and $1 - p$ for each population, the equilibrium phenomenon of the variant 2_c can be estimated by comparing these values with the corresponding observed frequencies as shown in table 3. It is of interest to note that the χ^2 value for each population is remarkably low. This suggests that the frequencies of the homozygotes and heterozygotes are maintained in a state of equilibrium in each of the three populations.

TABLE 2. *Sequences of 2_c in three populations*

	Tamiochang	Tangchiatze	Matisze
Bulb sequence (p)	0.38	0.69	0.64
Non-bulb sequence ($1 - p$)	0.62	0.31	0.36
Standard deviation	0.03	0.03	0.03

Comparison of the inversions in the three localities reveals that the "bulb" sequence vs. "non-bulb" sequence vary greatly from place to place. As shown in table 2, while the frequency of the bulb sequence is much higher than that of the non-bulb in the Tangchiatze and Matisze populations, the non-bulbed sequence is predominating over the bulbed in the Tamiochang population.

Moreover, it is of interest to note from table 2 that the frequency of a given gene arrangement, as expressed in terms of p and $1 - p$, may be reversed in different populations. By comparing the so-called commoner sequence, Philip (1942) believed that the frequencies of the gene arrangements were alike in the different populations studied by her. In the analysis of such cases where the frequencies of the opposite classes of homozygotes cannot be separately dealt with, the estimation of the proportions of the opposite arrangements cannot be followed with the aid of the preceding equation. Let the observed frequencies of the two homozygote classes together be $m(AA + BB)$, and that of the heterozygotes $l(AB)$, and let $l + m = n$. The expectations are:

$$n\{p^2 + (1 - p)^2\}, \qquad 2np(1 - p).$$

The logarithm of likelihood of the observation is:

$$L = m \log \{p^2 + (1 - p)^2\} + l \log 2p(1 - p).$$

Hence,

$$\frac{dL}{dp} = \frac{2m(2p - 1)}{p^2 + (1 - p)^2} + \frac{l(1 - 2p)}{p(1 - p)} = 0,$$

and

$$p^2 - p + \frac{l}{2n} = 0.$$

TABLE 3. *Estimation of the equilibrium for 2_c in the three populations*

	Tamiochang			Tangchiatze			Matisze		
	obs.	exp.	χ^2	obs.	exp.	χ^2	obs.	exp.	χ^2
Homozygous bulb	17	15.07	0.12	61	59.41	0.04	52	48.94	0.19
Heterozygous	46	49.79	0.31	49	52.15	0.19	48	54.11	0.69
Homozygous non-bulb	43	41.14	0.08	13	11.44	0.21	18	14.95	0.62
Total	106		0.51	123		0.44	118		1.50

TABLE 4. *Comparison of the commoner arrangements in the three populations*

	Tamiochang	Tangchiatze	Matisze
1a	0.95	0.91	0.96
1b	0.91	0.83	0.95
2a	0.96	0.88	0.96
2b	0.86	0.84	0.89
2c	0.62	0.69	0.64
3a	0.86	0.91	0.96

There must be two p values, when the equation is solved by actual numbers, the larger one representing the commoner gene arrangement in the population. The frequencies of the commoner arrangements of the six variants were accordingly calculated, and the values are shown in table 4. By comparing the values of the commoner arrangement among the three populations, the results do not show any significant departures in distribution. Even with the rearrangement 2_c, where relative frequencies of the two opposite arrangements are actually reversed between Tangchiatze and Matisze on one side and Tamiochang on the other, the values for the commoner arrangement in the three populations are nearly identical. It is, therefore, impossible to draw any conclusion concerning the parallel distribution of a chromosomal variation in different populations on the basis of the comparison of the commoner arrangement alone. As a matter of fact, even the calculation of the frequencies of the heterozygotes could reveal the difference which would otherwise be obscured by the comparison of the commoner sequence.

TABLE 5. *The $\Sigma\chi^2$ values of comparison of inversion heterozygotes between populations as expected by parallel distribution*

	Tamiochang-Tangchiatze	Tamiochang-Matisze	Tangchiatze-Matisze
1a	2.45	0.06	3.46
1b	3.24	1.93	10.33*
2a	7.08*	0.00	7.57*
2b	0.12	0.65	1.40
3a	1.55	9.37*	3.37

The frequency distribution of the heterozygotes in the three populations is therefore also compared in order to test for the validity of the assumption of parallel distribution. Table 5 represents the $\Sigma\chi^2$ values of the analysis. As indicated by the asterisks in the table, at least four cases show significant differences.

DISCUSSION

Of the six different chromosomal variants discovered in the natural populations of *Chironomus spec.*, the tandem inversion (2b) and the bulb-like puff structure (2c) are of especial interest. As far as we know, a tandem inversion has never been reported before in a free-living population. It has apparently resulted from the simultaneous occurrence of four separate breaks in a single arm of a chromosome. Previous to this finding, the general belief concerning chromosomal mutations in nature was that each event does not involve more than two breaks at a time, and that complex chromosomal rearrangement occasionally found in nature represents accumulation of successive changes. The present finding lends support to the opposite view that complex chromosome rearrangement can arise by a single step.

In appearance, as well as in position, the bulb-like variation is similar to the large "puff" region found in one of the chromosomes in the hybrid between two Palestinian Chironomids (Goldschmidt, 1942). Although its specificity is beyond any doubt, the nature of the difference from its homologue and the mechanism of its origin are not at all clear. Nevertheless, it is obvious that this is not an ordinary type of chromosomal change that involves only chromosomal breakage and reunion. Judging from the staining reaction, this looks more like a chemical change than a mechanical one.

As shown by the results of our analysis, the six chromosomal variants appear to combine at random in each of the

three populations studied. Some of the inversions involved are found on the two separate arms of the same chromosome, namely, 1a and 1b and 2a and 2b, and in one case, namely, 2a and 2c, the two inversions are even located so closely together that only a few bands intervene in the salivary gland chromosome preparations. While free recombination of inversions in different arms may be explained by the relatively long distance between them, the independence of the two closely localized inversions requires some additional consideration. In this connection, it is interesting to recall the calculation of Robbins (1918) who found that in an ideal population the distribution of two pairs of genes, whether independent or linked, and whatever the original proportion of the gametes may be, can reach a so-called "fixed" condition with a definite rate under random mating system. The rate at which two linked genes reach a state of equilibrium, that is the "fixed" condition of Robbins, depends upon the amount of crossing-over between them; the higher the crossing-over frequency, the faster will it reach equilibrium. Once equilibrium is established, the crossing-over products of the repulsion phase Ab/aB will compensate those of the coupling phase AB/ab. Hence, from the random sample it becomes difficult to observe the linkage phenomenon of two genes that are actually linked together. Since inversions located in a single chromosome must behave like linked genes, the above explanation should be valid for our case.

Although the distribution of the six inversions is maintained in an equilibrium state within each of the three populations, the relative frequencies of these inversions are different in different populations. The case of bulb-like variation is especially interesting, as has been described above. That the three populations found in three rather closely adjacent localities exist at different levels of equilibrium suggests that they are at least partially isolated geographically. In view of the general weakness of flight of the adult *Chironomus,* it appears probable that this may be due to the low migration power of this form.

Acknowledgments

The authors wish to express their hearty thanks to Professor Th. Dobzhansky of Columbia University and Professor C. C. Tan of Chekiang University for their valuable suggestions and criticisms and their kindness in reading the manuscript.

Summary

Using the salivary gland chromosome technique, three neighboring populations of *Chironomus* were analyzed for the incidence of chromosome rearrangements.

In all three populations, there were found six major intra-chromosomal rearrangements. Four of them are simple paracentric inversions, one paracentric tandem inversion and one "bulb"-like puff structure corresponding to four heavily stained bands in its homologue.

The different variations are combined at random regardless of whether they are located in separate chromosomes, or in separate arms of a chromosome, or on the same arm of a chromosome, indicating that all of the three populations are maintained in an equilibrium state.

The relative frequencies of the variants are, however, different in different populations. This suggests that the three populations, though separated from each other by only a few miles, are at least partially isolated.

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THE BROMELIAD–ANOPHELES–MALARIA COMPLEX IN TRINIDAD. I–THE BROMELIAD FLORA [1, 2]

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Received January 26, 1948

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1. INTRODUCTION AND GENERAL SUMMARY

Downs and Pittendrigh (1946) have given a general account of the malaria problem in Trinidad, British West Indies, which is due to *Anopheles* (*Kerteszia*) *bellator* D. and K. This mosquito and all but one of the other members of the anopheline subgenus *Kerteszia* breed exclusively in the water held between the leaves of epiphytic bromeliads. The likelihood that these bromeliad-breeding anophelines were involved in some instances of malaria transmission was appreciated 45 years ago by Lutz (1903) in Sao Paulo. It was not until 1942, however, that their public health importance was fully established by Rozeboom and Laird, and by Downs, Gillette, and Shannon (1943), who reported natural infections of *Plasmodium* in *Anopheles* (*K.*) *bellator* in Trinidad.

The present paper, and one to follow, consists primarily of an ecological study that was undertaken initially to elucidate the factors responsible for two striking epidemiological features of the malaria in Trinidad transmitted by the bromeliad-breeding anophelines. These two features are as follows.

(A) The malaria concerned has a well defined distribution, being restricted to the wetter part of the island. Within its overall range its intensity shows a very clear positive correlation with rainfall level (Downs and Pittendrigh, 1946). Moving across the island from northeast to southwest, there is a simultaneous drop in both rainfall and malaria intensity but there is, on first analysis, no comparable decrease in the size of the epiphytic bromeliad flora which comprises the mosquitoes' breeding ground.

(B) The species of anopheline vector varies geographically. Throughout most of the range in which "bromeliad-ma-

[1] The studies on which this paper is based were done under the joint auspices of the Trinidad Government and the International Health Division of the Rockefeller Foundation.

[2] A contribution of the Rockefeller Foundation toward the cost of printing is gratefully acknowledged.—ED.

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laria" occurs, it is transmitted by *Anopheles bellator* although both this species and *Anopheles* (*Kerteszia*) *homunculus* Komp are common in the forests. However, in the wettest valleys in the northeast of the island, where malaria is present, *bellator* is absent and *homunculus* is not only generally abundant but is found biting man near houses. This is never seen elsewhere. A search for natural infections of *Plasmodium* in these *homunculus* populations has not been made but from the epidemiological evidence there can be no doubt of its vector status in these areas.

A further, purely practical, aim of the work which led to an interesting theoretical result, was to delimit as closely as possible the distribution of anopheline breeding in the extensive bromeliad floras both with respect to plant species utilised and height above ground. This had an obvious bearing on any malaria control program based on the destruction of breeding places.

Results of evolutionary significance. An analysis has been made essentially in terms of the differing climatic requirements of the two anopheline species and the bromeliad flora they are dependent on. The principal results are of interest to the evolutionary biologist. They are as follows.

(1) It is found that the epiphytic bromeliad flora in forest is, as a whole, characterised by a very strong light demand which is responsible for its concentration in the higher levels of forest. This demand for high light intensities reflects the evolutionary history of the Bromeliaceae as originally a desert group that has subsequently become highly successful in the upper levels in rainforest. Their success in xeric epiphytic habitats is due essentially to preadaptations developed as desert saxicoles.

(2) *Anopheles* (*K.*) *bellator* D. and K. and *Anopheles* (*K.*) *homunculus* Komp are very closely related species. The only usable morphological difference between the adults is the relative amount of white on the tarsal segments of the hind leg. They differ in their microclimatic requirements in such a way as to suggest that they evolved allopatrically in regions differing primarily in humidity. In those regions where their ranges overlap —such as the greater part of central Trinidad—they are spatially segregated on the vertical gradient of humidity in forest, and they also differ in the timing of their major peak of feeding activity. In their oviposition activity they are still more strongly isolated on the gradient of forest microclimates than they are as feeding adults. *A. homunculus* manifests a demand for higher humidities than *bellator* in being much lower on the forest profile. The limitation of *homunculus* oviposition activity to the lower, moister levels in forest imposes a severe restriction on the size and specific composition of the bromeliad flora available to it for its breeding. It is found experimentally that, correlated with this restriction, there is, comparing the two anophelines, a greater ability on the part of *homunculus* to utilise the specialised group of plant species available to it. The likelihood that this correlation is due to selection is examined and the consequences discussed.

(3) Associated with the relatively rigid light demand of the epiphytic bromeliad flora is the fact that its vertical distribution on the profile of cacao plantations does not shift even in areas of extremely different overall humidity. The reverse holds for the mosquitoes: humidity is their prime ecoclimatic variable. Decrease in the general humidity level leads to a downwards shift on the forest profile and this can be shown to account for the positive correlation of their density with rainfall level. Thus, such a downwards movement on the forest profile will divorce the mosquito from its breeding ground which has a fixed vertical distribution. The geographic variation in vector status of *bellator* and *homunculus* and the absence of malaria in the drier sections of the island are analyzed in these terms. The key to the main features of the geographic distribution of both the anophe-

lines and malaria is given by the microdistribution of the two species on the vertical gradient of humidity in forest.

The mosquito data will be presented later. The present paper is an ecological discussion of the bromeliads which is essentially no more than a descriptive account of the ecological types encountered in the family and their distribution both geographically and in varied forest microclimates. The main purposes of the work on the plants were (1) to determine their vertical distribution in forest and to relate this to the vertical distribution of mosquito oviposition activity; and (2) to determine whether or not there was any geographic variation in the flora adequate to account for the geographic variation of the mosquito population.

The majority of the work was done at a station for field studies in Tamana in the central part of Trinidad (fig. 1).

The Nomenclature of the Bromeliads

The following change in nomenclature has to be made. **Thecophyllum Splitgerberi** (Mez) comb. nov. *Guzmania Splitgerberi* Mez in DC. Monogr. Phaner. IX. (1896). The petals are ligulate, and do not show the overlapping-connate condition that characterizes *Guzmania*. The form of the inflorescence, in which the primary bracts equal or exceed the spikes they subtend, places the species in *Thecophyllum* rather than *Vriesia*.

The generic name *"Gravisia"* is retained for *Gravisia aripensis* N. E. Brown although this species certainly does not belong in the genus to which Brown assigned it: the pollen has four pores. However, the confused state of generic boundaries in the Aechminae precludes a worthwhile re-assignment at present.

Names of bromeliads in this text have not been encumbered with citations of authors except in the figures. The nomenclature used is that given by Broadway and Smith (1933) except for the following changes subsequently published by Smith from 1934 onwards in the Contributions for the Gray Herbarium of Harvard University: *Vriesia stenostachya* (Bak.) Mez becomes *Vriesia glutinosa* Lindl.; *Hohenbergia oligosphaera* (Bak.) Mez becomes *Hohenbergia stellata* Schult. f.; *Ananas sativus* Schult. f. becomes *Ananas comosus* (L.) Merrill; the Trinidad plant given in 1933 as *Catopsis nutans* (Sw.) Griseb. is *Catopsis floribunda* (Brongn.) L. B. Smith.

In the course of the present studies the following species have been added to the list given by Broadway and Smith: *Vriesia platynema* Gaudich.; *Vriesia amazonica* Mez; *Thecophyllum Johnstonei* Mez; *Thecophyllum capituligerum* (Griseb.) Smith; *Thecophyllum Splitgerberi* (Mez); *Guzmania sanguinea* Andre; and *Aechmea* sp. nov.

It is a pleasure to acknowledge here the assistance the writer has received in taxonomic matters from Dr. Lyman B. Smith of the Smithsonian Institution of Washington.

2. Topography and Climate

Trinidad is a fairly large island approximately fifty miles from north to south by thirty-five from east to west. It lies at the southernmost end of the Antillean chain, 10°30′ north of the equator. It is, however, geologically part of the parian subcontinent (Schuchert, 1935) and has no affinities with the Antilles. Its flora and fauna are almost exclusively continental South American.

The principal topographic features of the island are given in figure 1. There is (1) a Northern Range of Mountains running parallel to the north coast. To the south there is (2) the flat Sangre Grande basin, and (3) the Central Range of hills. Tamana, where the bulk of the studies reported in these two papers were made, is located in the northeast section of the Central Range on the eastern slope of Mount Tamana itself which is 1009 feet high. (4) There is a second flat region south of the Central Range, and finally (5) the Southern Range of low hills.

The climate of the island has been extensively described in several earlier publications (Bain, 1934; Marshall, 1934; Beard, 1946) and it is unnecessary here to note more than the essential features. The climate is on the whole very equable, although there is well-marked variation in the rainfall both seasonally and geographically. The geographic distribution of rainfall is shown in figure 1. It is determined by the direction of the Northeast Trades and the presence of the Northern Mountain range.

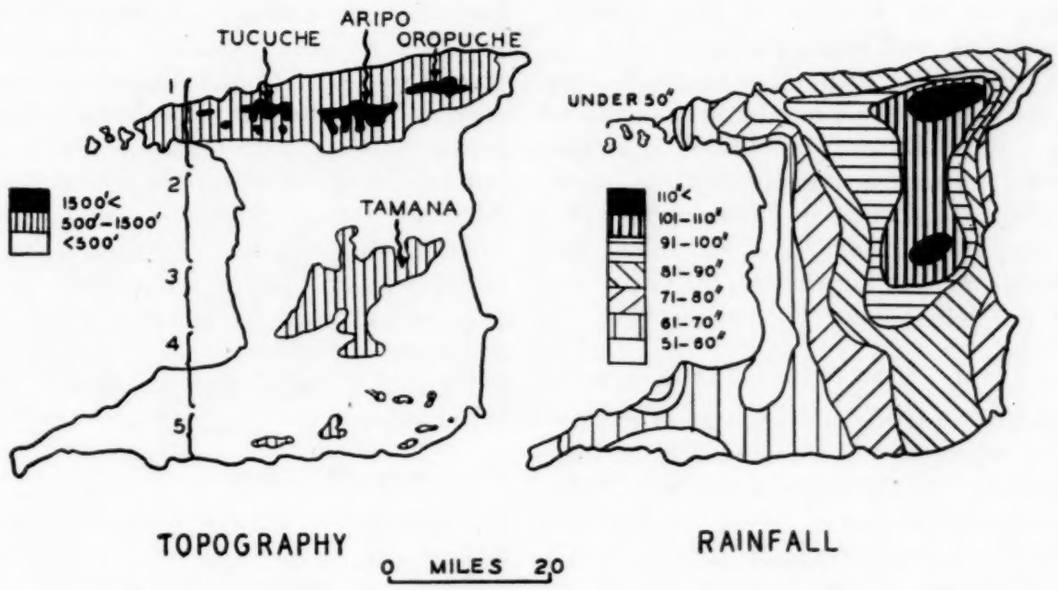


FIG. 1. The topography and geographic distribution of rainfall in Trinidad, B.W.I. (1) The Northern Mountain Range; (2) Sangre Grande Basin; (3) The Central Range of Hills; (4) Princes Town Flat; (5) Southern Range of Hills.

A major dry season occurs from February to April, and a major rainy season from May through August. September and October are drier, the season being locally known as "petite careme." November and December constitute a second, minor, wet season. In Tamana the dry season is very slight, the lowest monthly precipitation being as high as 3.2" in 1945.

Temperatures throughout the island are moderate and very stable. Mean minima are in the neighbourhood of 68° F. to 72° F., and mean maxima from 86° F. to 89° F. The mean daily range is therefore about 17 degrees. The hottest months are in the major rainy season but there is extremely little seasonal variation.

Humidity maxima throughout the island are close to complete saturation. In Port of Spain dry season minima are approximately 50 per cent RH, and wet season minima 60 percent RH.

In discussing the distribution of the bromeliads and the mosquitoes, seven climatic zones have been recognised and designated for convenience of reference in the text by the letters A through G. Their delimitation is based primarily on rainfall.

The *A Zone* includes the peaks above 2500' in the Northern Mountain Range. It is therefore very small, indeed, only a fraction of the areas given in solid black in the topographic map in figure 1. The peaks concerned are virtually in perpetual rain which must add to well over 250" per annum. Beard (1946) gives the temperature range here as 59°–65° F. and states the evaporating power of the air is high in spite of the high rainfall. This is presumably due to the almost incessant high wind.

The *B Zone* is a well-defined region of true montane forest lying immediately below these peaks. It comprises the uppermost reaches of the Platanal, Aripo, Guanapo, and Caura Valleys, all of which are included in or immediately adjacent to the black areas in the topographic map. Here again the rainfall is extremely high, probably in the neighbourhood of 200". Beard gives the approximate temperature range as 62°–68° F.

The *C Zone* comprises two areas. The first lies to the south and east of the B

areas on the foothills of the northern mountains, well below the black areas on the topographic map. The second C area is the eastern slope of Mount Tamana in the Central Range (fig. 1). The annual rainfall as recorded at our field station in Tamana (C Zone) in 1944–5 was 125".

The *D, E, F, and G Zones* follow the rainfall map as follows: D, 90"–110"; E. 70"–90"; F, 50"–70"; G, under 50".

3. Vegetation Types

Beard's (1946) full account of the natural vegetation of Trinidad may be consulted for details of the extent and distribution of the various forest types in the island. Approximately 50 percent of the total acreage of the island is still under natural forest vegetation. A further 20 percent is under cacao plantation which also supports a very heavy epiphytic bromeliad flora.

(*a*) *Forest*

The forests referred to throughout this paper are of the "rain forest" type. The specific association is *Carapa Guianensis–Eschweilera subglandulosa.* This is by far the commonest forest type in Trinidad. It is in general three storied but the stratification in our experience has never been sharply defined. The canopy proper is broken and somewhat open, varying from 40′ to 100′. Above the canopy is a highly discontinuous stratum of emergents; and below it is a lower stratum from 10′ to 40′. There is also a fairly well developed ground society of herbs and tree seedlings. Epiphytes and lianes are exceptionally well developed.

Marshall (1934) considered *Carapa–Eschweilera* as true rain forest. In his recent discussions, however, Beard (1946) has adopted a more restricted view of rain forest and classifies the Trinidad *Carapa–Eschweilera* as Evergreen Seasonal Forest. This author notes in connection with *Carapa–Eschweilera* forests that (1) their rainfall is markedly seasonal and (2) a very small proportion of the species show some seasonal leaf fall. He maintains also that there is a structural difference between Evergreen Seasonal Forest and true Rain Forest: the former is more open. The forests in Tamana where the present studies were made are the faciation of *Carapa–Eschweilera* which Beard describes as *Pentaclethra macroloba–Maximiliana elegans.* In this faciation Beard lists 95 species. His 1946 paper may be consulted for floristic detail and statistics.

Figures 6 and 7 give profiles of this forest type.

(*b*)–*Cacao Plantation*

Considered as a forest type, cacao plantations (fig. 3 and 7c) offer a radically different structure from the *Carapa–Eschweilera* association described above. The cacao trees (*Theobroma cacao*) are planted twelve feet apart and form a more or less closed canopy at fifteen to twenty feet above ground. *Erythrina micropteryx,* which is known as the Bocare Immortelle in Trinidad, is interplanted as a shade tree every twenty-five feet. Actually the Immortelles offer very little shade as such: they have very characteristic long, tenuous, wide-spreading branches which are very sparsely foliated and therefore never form a closed canopy even within the individual tree which reaches a height of 50′–80′. Figure 3 shows the very open nature of the Immortelle stratum.

In mountain areas *Erythrina glauca,* the Anauca Immortelle, replaces the Bocare. It is a much taller tree, reaching commonly to 100′, with a stricter branching habit and a still sparser foliation than that of the Bocare.

(*c*) *Internal Climate of Forest and Cacao Plantation*

The stratification of the internal climate in forest has been studied by several workers, notably Allee (1926). More recently Bates (1945) has given a general account of the topic. Well-marked microclimatic gradients exist from the forest floor to the canopy. The lower levels of

TABLE 1. *Temperature and humidity gradients throughout the day in Carapa-Eschweilera forest (August, wet season, 1945)*

Height in feet	A.M. 10	11	12	1	2	3	4	5	6	7 P.M.	Range
	Relative humidity (in %)										
65	91	94	91	88	88	85	93	97	100	100	15
45	90	91	94	88	89	94	94	98	99	100	12
25	95	93	96	94	91	98	95	99	100	100	9
5	99	95	98	97	99	99	99	100	99	97	5
Range	9	4	7	9	11	14	6	3	1	3	
	Temperature (in °F.)										
65	80	79	80	81	81	81	80	79	78	77	4
45	79	78	80	81	80	79	80	79	78	77	4
25	77	78	79	80	80	79	79	79	78	78	3
5	77	78	79	79	79	79	79	78	77	78	2
Range	3	1	1	2	2	2	1	1	1	1	

forest are less windy, cooler, darker, and more humid than the upper levels. The daily (and presumably the seasonal) range of all these climatic features is smaller in the lower levels than in the upper levels which are more exposed to the variations of the external climate. Table 1 gives temperature and humidity data for four levels in the forests on August 1, 1945 (Tamana). The vertical gradient of decreasing humidity and increasing temperature is clear; and also the fact that the range of variation for both features is larger in the upper levels.

TABLE 2. *Sample relative humidity gradients in forest (F) and cacao plantation (C)*

Ht. in feet	Dry season				Wet
	12. IV. 45		30. IV. 45		1. VIII. 45
	F	C	F	C	F
75	76		83		
65	77		84		90
55	78		84		
45	79	72	87	76	91
35	79	72	88	76	
25	81	74	85	76	94
15	82	75	87	78	
5	84	77	88	80	99

Inasmuch as the constancy of the lower levels is due to the protective effect of the higher ones, it is clear that the climate in lower and more open forests will be as a whole more variable, drier, lighter, and hotter than the climate in higher, close-structured forest. This effect is seen in Table 2 where data are given for humidity during comparable observation times in the *Carapa-Eschweilera* forests and a cacao plantation 300 yards away. The climate is drier in the plantation.

4. ECOLOGICAL TYPES IN THE BROMELIACEAE

The Bromeliaceae is a large * and well defined family of monocotyledonous plants. With the exception of a single West African species it is entirely restricted to the tropics and subtropics of the new world. The family as a whole is characterized by its tendency towards xerophytism and the remarkable absorbing system of the leaves that is associated with this habit.

Three sub-families are commonly recognized. The Pitcairnioideae contains

* Over 1600 species are known. There is little doubt that the family comprises at least 2000.

thirteen genera, and is entirely terrestrial. The Tillandsioideae (six genera) is almost entirely epiphytic. The Bromelioideae (thirty genera) contains many terrestrial forms but is predominantly epiphytic (Harms, 1930; Mez, 1935).

The most primitive *Bromeliaceae,* the Pitcairnioideae, are terrestrial plants of a xerophytic nature with a well developed root system. Their leaves play little or no part in water or mineral absorption. In the higher sections of the family the root system plays a progressively smaller part in the plants' absorptive activities, and this is accompanied by the evolution of a system of epidermal trichomes on the leaves which come to occupy a position of central importance in the plant's overall water and nutrient economy. A. F. W. Schimper (1888) was the first to appreciate the main features of this unusual absorptive system in the higher Bromeliaceae. Mez (1904) subsequently made on extensive study of the structure and physiology of the epidermal absorbing trichomes for which he introduced the term *trichompompe.*** Mez's conclusions are too elaborate to be reviewed here—suffice it to say that the absorbing scales when desiccated collapse in such a way as to exclude water-loss through them, and when wetted expand in such a way as to set up first a suction pump mechanism drawing water into a dead cell apposed to a stalk of living cells through which the water is then drawn osmotically to the mesophyll. The absorbing scale is thus effectively a one way valve, and its evolution has proved one of the two keys to the overwhelming success of the family as epiphytes. The other is the habit of impounding water and humus between expanded leaf bases and in this way retaining a soil equivalent in direct contact with the foliar absorbing system.

In the following paragraphs a description is given of what are considered four basic ecological types within the family. An appreciation of their features is necessary for any discussion of their distribution and the factors affecting it. At the end of a discussion of the morphology of the absorbing scale in various genera in the family, Tietze (1906) attempted a classification of types within the Bromeliaceae. He based this partly on the published observations of Mez and Schimper, and partly on inference from the morphology of herbarium material. He recognized four groups as follows: (a) Terrestrial plants of a low order; (b) Terrestrials of a high order; (c) Epiphytes of a low order; and (d) Epiphytes of a high order. The classification developed here coincides in a large part with Tietze's and owes much to him. There are, however, several points of divergence.

First, Tietze's groupings relate specifically to the habitat of the plant: he recognizes terrestrials or epiphytes. This is not the basis of the present classification. The fundamental cleavage in the family is between plants dependent on their substratum for nutriment and those that are independent of it. Several lines within the family contain both saxicoles and epiphytes, and indeed many species are facultatively the one or the other. The habitats differ but the plants share in common independence of the substratum.

Secondly, Type II as described below is wholly unrecognized as such by Tietze. To be sure, the two genera referred to here as Type II are included under Tietze's large second group, but it is unknown whether the other genera in his "Terrestrials of a higher order" belong under Type II or are primitive cases of Type III. Tietze's criteria are wholly different from those employed here.

The classification adopted here also attempts a more detailed breakdown of the four major types, which are listed with their Trinidadian representatives in table 3. They will now be considered.

Type I: Soil Root

This type includes those plants which on taxonomic grounds are considered the

** An easily available account in English of the detailed structure of bromeliad absorbing scales will be found in Haberlandt (1914).

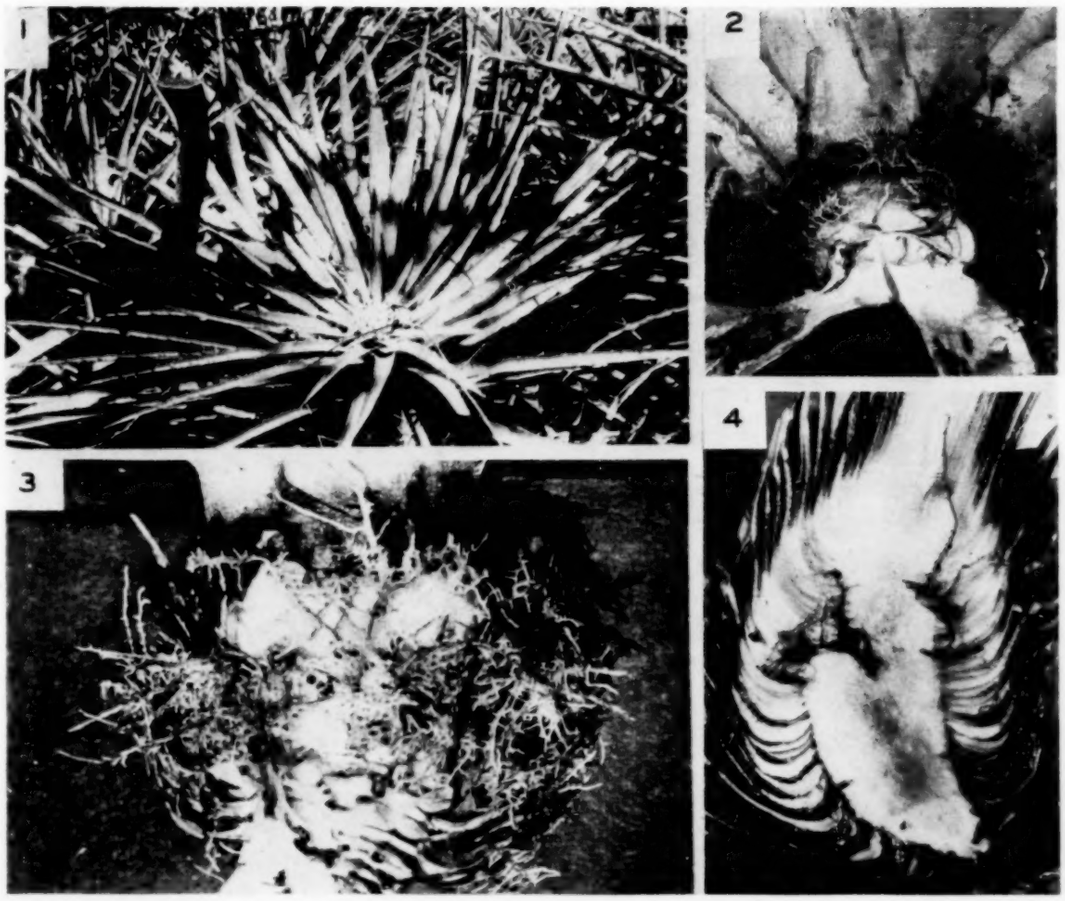


FIG. 2. *Bromelia humilis* Jacq. in the *Acacia*-cactus scrub on the coast of the Paria Peninsula, Venezuela. A Type -II (tank-root) bromeliad.

2 (1) The intact plant in flower, growing on sand. Note the gutter-like shape of the leaf blades which drains water into interfoliar niches.

2 (2) The outermost leaves have been flexed back to show the interfoliar (mycotrophic) roots which exploit the humus that collects in the interfoliar niches.

2 (3) A number of leaves have been removed entirely to show the extent of the interfoliar root system. Note the band of dark brown epidermal trichomes at the base of the remaining leaves above the roots.

2 (4) A longitudinal section through the plant from which the leaf blades have been cut away. Two of the interfoliar roots have been withdrawn from between the leaves to show their axillary origin.

FIG. 3. *Gravisia aquilega* (Salisb.) Mez, a Type IIIb bromeliad epiphytic on *Erythrina micropteryx* in Trinidad.

3 (1) A cacao plantation with the cacao canopy (*Theobroma cacao*) in foreground. *Erythrinas* in background supporting large numbers of bromeliads; the large clumps are *Gravisia aquilega*.

3 (2) A large group of *Gravisia aquilega* on *E. micropteryx;* cacao in foreground.

3 (3) A single specimen of *Gravisia aquilega* removed from the *Erythrina*. Note the humus and water splashed from the interfoliar tank onto the leaves in the process of removing the plant.

3 (4) The same specimen with leaf blades cut away to show the overlapping leaf bases that impound the water and humus. Decaying leaves and other vegetable matter are clearly seen. The dark brown color of the leaf surfaces in this basal region is due to the covering of epidermal absorbing trichomes.

FIG. 4. Type III bromeliads (cf. table 1)

4 (1) *Wittmackia lingulata* (L.) Mez, a strongly xerophytic tank-nest (IIIb) species with a myrmecophilous tendency. Note accumulated dead leaves. Leaves 1 meter long.

4 (2) *Vriesia simplex* (Vell.) Beer, a type IIIc species with poorly developed tank. Leaves 20 cm. long.

4 (3) *Vriesia amazonica* Mez. Type IIIa. Leaves ca. 1 meter long.

4 (4) *Aechmea Mertensii* (Meyer) Schult. fil. (type IIId) in an ant-garden. Note the massed roots at the base surrounding the actual ant nest which is also penetrated by the roots. At the left are leaves of *Anthurium* (*?gracile* Lindl.) which is also a regular member of the

Tamana district ant-gardens. At the lower right there are three of the grass-like leaves of the orchid, *Hexisia reflexa* Rchb. f., which is also part of the garden. A gesneriad (*Codonanthe* sp.) is in the garden but at the rear. Leaves 30 cm. long.

4 (5) *"Gravisia" aripensis* N. E. Brown at lower left; type IIIa. Leaves up to 1 meter long. Young plants of *Glomeropitcairnia erectiflora* Mez upper right; type IIIa. Leaves slightly over 1 meter long. Both epiphytic in the wet *Clusia intertexta* "elfin woodland" (cf. Beard (1946)) on El Cerro del Aripo (cf. fig. 1). Batrachians were breeding in the *G. erectiflora*.

4 (6) *Aechmea dichlamydea* Bak. Type IIIa. Leaves 90 cm. long.

4 (7) *Aechmea nudicaulis* (L.) Grisebach on *Erythrina micropteryx*. Type IIIa. Leaves 40 cm. long.

4 (8) *Vriesia platynema* Gaudich. Type IIIb. Removed from tree. Note the great accumulation of humic material not only between individual leaves but between the separate rosettes. This material is exploited by secondary (mycotrophic) roots. Leaves ca. 1 meter long.

4 (9) *Vriesia procera* (Mart.) Wittmack. Type IIIa. Removed from tree. Leaves ca. 30 cm. long.

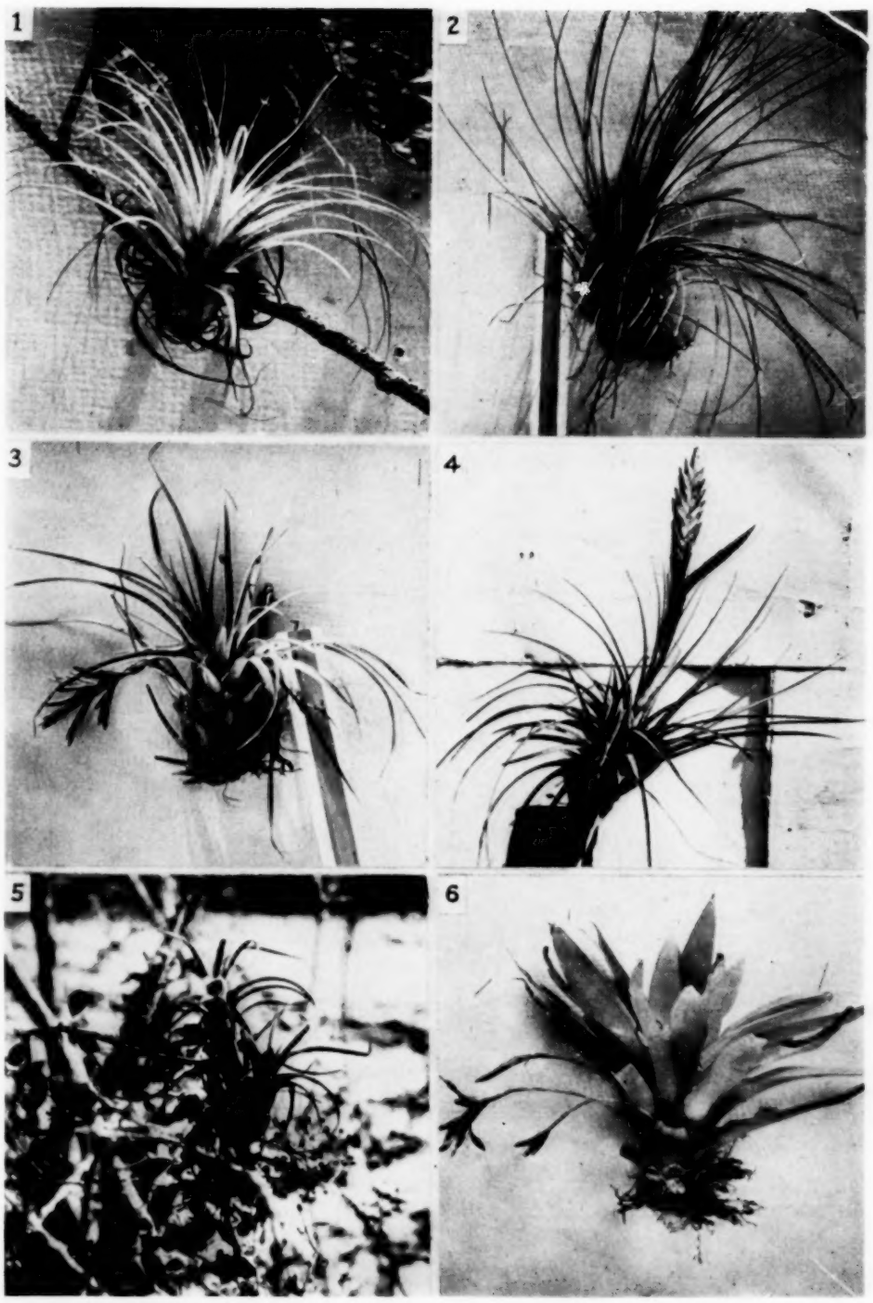


FIG. 5. Type IV Bromeliads (except 5 (6))

5 (1) *Tillandsia Gardneri* Lindl. Type IVb. Epiphytic on *Theobroma cacao*. Note the outermost leaves are reflexed and curled around the branch and old decayed leaves at the base of the plant (cf. p. 68). Leaves 12 cm. long.

5 (2) *Tillandsia juncea* Ruiz et Pav. Type IVb. Leaves 25 cm. long.

5 (3) *Tillandsia didistichoides* Mez. Type IVa. Leaves 17 cm. long.

5 (4) *Tillandsia fasciculata* Swartz. Type IVa. Leaves 40 cm. long.

5 (5) *Tillandsia flexuosa* Lindl. Type IVc. Leaves ca. 25 cm. long.

5 (6) *Tillsandsia complanata* Benth. Type IIIa. Leaves ca. 25 cm. long. Note the dry wiry roots that only serve the mechanical function of gripping the plant to a tree.

TABLE 3. *A classification of ecological types in the Bromeliaceae with Trinidadian representatives of the several groups.* (N) = a slight tendency towards the nest condition.
(Myr.) = sometimes myrmecophilous

TYPE I. SOIL—ROOT
Pitcairnia integrifolia

TYPE II: TANK—ROOT
(*a*) *Soil and Tank—Root*
Bromelia Karatas; B. chrysantha; Ananas comosus
(*b*) *Tank—Root, only or predominantly*
Bromelia humilis

TYPE III: TANK—ABSORBING TRICHOME
(*a*) *Tank—Absorbing Trichome only*
Tillandsia triticea; T. micrantha; T. complanata; T. rubra; Vriesia procera; V. albiflora; V. amazonica (N); *V. glutinosa; Thecophyllum Johnstonei; Th. Splitgerberi; Th. capituligerum; Guzmania sanguinea; G. monostachia* (N); *Catopsis floribunda* (N); *C. sessiliflora; C. Berteroniana; Glomeropitcairnia erectiflora; Aechmea nudicaulis* (Myr)
(*b*) *Tank—Absorbing Trichome* (and *Nest-Root* secondarily)
Vriesia platynema; V. macrostachya; V. Broadwayi; Aechmea sp. nov.; *Ae. dichlamydea; Ae. porteoides; Ae. bromeliaefolia; Gravisia aquilega; Hohenbergia stellata; Gravisia aripensis; Wittmackia lingulata* (Myr)
(*c*) *"Ephemeral" Tank*
Tillandsia monadelpha; T. anceps; Vriesia longibracteata; V. simplex; Guzmania lingulata
(*d*) *"Myrmecophilous" Tank*
Tillandsia bulbosa; Aechmea Mertensii (Ant-Gardens); *Araeococcus micranthus* (Ant-Gardens)

TYPE IV: ATMOSPHERE—ABSORBING TRICHOME
(*a*) *Tank and Atmosphere—Absorbing Trichome* ("Intermediates")
Tillandsia fasciculata; (T. canescens); T. utriculata; T. didistichoides; T. subimbricata
(*b*) *Atmosphere—Absorbing Trichome*
(1) Rain Type (see Mez (1904))
Tillandsia stricta; T. pulchella; T. juncea
(2) Dew Type (see Mez (1904))
T. Gardneri; T. usneoides
(*c*) *Myrmecophilous Atmospheric*
Tillandsia flexuosa

most primitive in the family: the Pitcairnioideae. The soil constitutes their supply of water and mineral nutrients which are absorbed by a fully developed root system. The leaves bear epidermal trichomes of a low degree of organisation (Tietze). There is no experimental evidence that these scales serve any other function than the avoidance of excessive transpiration (Smith, 1934). However, they do have a definite stalk of cells below them which pierces the cutinised epidermis and thus leads directly from the external scale to the mesophyll. As Harms (1930) notes, this is suggestive of an absorptive function and it is presumably the basis of a wholly unsupported statement by Mez (1904, p. 159) that the trichomes "which take up water appear . . . even in the Pitcairnioideae" (transl.). The bases of the leaves are not expanded and retain no water between themselves. *Pitcairnia integrifolia* is the only Trinidadian representative. It seems very likely that the whole of the Pitcairnioideae belong under this type.

Type II: Tank-Root

Four species: *Bromelia humilis, Bromelia Karatas, Bromelia chrysantha,* and *Ananas comosus,* are known to belong here. They exhibit to greater or less extent the peculiar habit that characterizes the type, which has been previously unrecognized in the family.

Bromelia humilis (fig. 2) is the most typical of the four species. This is a common plant forming extensive societies on the rocks and loose gravel of the coastal fringe in the northwestern peninsula of Trinidad, in the Paria Peninsula of Venezuela, and in the islands between the

peninsulas. These societies of *Bromelia humilis* are often found to consist of numerous leaf rosettes connected to one another by dried stolons. Roots enter the ground only rarely and the whole mat of rosettes can usually be simply lifted from the rocks or gravel on which it lies, and from which it is evidently wholly independent for nutrition or water supply.

The leaf bases are expanded and overlap, and in this way form a series of niches which retain rainwater and dew. The water that collects in these spaces between leaf bases is usually dirty and contains numerous insect larvae as well as decaying leaves that have dropped on to the plant. It is this water and its humic content which constitutes the plant's nutrient supply, and this is exploited by a rich system of axillary roots which grow upwards between the overlapping leaf bases to reach the water.

Figure 2 (1) shows a specimen of *Bromelia humilis* in which several of the leaves have been pulled back to show the up-growing roots. The specimen in figure 2 (3) has had all of its leaves removed to show the extensiveness of the interfoliar roots.

The root system is of interest in another way: it is a mycorrhiza. The fungus is present within the root hairs and other epidermal cells, but the bulk of the mycelium is ectotrophic. While no specific role can safely be attributed to a mycorrhiza without experimental study, it does seem likely that its presence here may be related to the purely organic nature of the nutrient supply in the interfoliar niches. This idea gains support from the conditions found in the other three members of Type II.

Bromelia Karatas, B. chrysantha, and *Ananas comosus* have also been found showing the interfoliar root system described for *B. humilis.* None of these latter three, however, are exclusively dependent on the interfoliar root system, since they have in addition abundant roots penetrating the soil. In *B. Karatas* and *B. chrysantha* the interfoliar roots are in fact somewhat rare, and some individual plants lack them entirely. In "wild" specimens of *Ananas comosus* in Trinidad, however, the interfoliar root system has always been present and extensive in the writer's experience.

In all three species the interfoliar roots are thick mycorrhizae but the main root system that penetrates the soil is apparently either entirely non-mycotrophic or only very weakly so. This is in conformity with the notion that the mycorrhiza is associated with the completely organic nature of the nutrient supply it exploits.

In all four species there is a heavier concentration of epidermal trichomes on the leaf sheath than is found in the terrestrial plants of Type I. In *Bromelia humilis* the scales form a dense mat of capillaries in which the axillary roots are imbedded. Figure 2 (2) shows this mat of dark brown trichomes. In specimens of *humilis* that were examined at the height of the dry season (April) in Venezuelan coastal desert scrub there was little or no water in some of the interfoliar niches, but the mat of trichomes was still wet. Evidently the system of capillaries created by the thick layer of trichomes resists desiccation and in this way protects the roots it surrounds. The roots themselves do not reach the open water-retaining niche between the leaves; the band of epidermal trichomes serves to conduct the water from above to the roots below (fig. 2 (2) and 2 (4)). It is highly probable that in the species of Type II the epidermal scales have a regular water absorbing function. In all of them there again is the presumptive evidence of a definite stalk of cells leading from the external scale to the mesophyll, and Aso (17) has demonstrated that the scales in *Ananas* (Type II) do absorb water although not mineral solutes.

Type III: Tank-Absorbing Trichome

The great majority of bromeliads fall within this group. The type is characterized by the further expansion of the leaf

bases to hold greater quantities of water within the interfoliar niches which may, for convenience, be collectively referred to by Schimper's word, "tank." Schimper (1888) and Tietze (1906) have described anatomical features associated with the tank habit. The most notable of these is an increase of mechanical support within the leaves which, being rosulate and upright, effectively constitute a funnel which collects rain and falling leaves.

Roots, which played the major or exclusive part in absorption in Types I and II, are here predominantly or exclusively mechanical in function. They serve to anchor the plant to the tree or rock on which it rests. The vascular system is greatly reduced and obscured by the development of mechanical tissue which gives the roots their hard wiry structure.

Absorption of water and nutrients takes place through the agency of the epidermal trichomes (absorbing scale). In most species these are restricted to or at least concentrated on the leaf bases that impound the water. They have a much more elaborate structure than in the two previous types and have been firmly established by Schimper (1888) and Mez (1904) as absorbing organs.

The most striking feature of the tank bromeliads is the huge size that some of them attain in spite of their strictly epiphytic habit. The inflorescence of *Glomeropitcairnia erectiflora* in Trinidad may attain nine feet in height. Mature plants of this species when full of water are sometimes too heavy for a single man to lift. Five liters is not an abnormal content of free water for the interfoliar niches of *G. erectiflora*. This success is due essentially to the fact that they are, at least as mature plants, quite independent of any "external" nutrient supply. The tank constitutes the plant's own, potentially limitless, soil-equivalent; it not only collects all the water that is necessary but also quantities of fallen leaves and thereby attracts an immense aquatic fauna and flora which live in the interfoliar niches.

Figure 3 is a photograph of a species of Type III, *Gravisia aquilega*. The leaf blades have been cut away (fig. 3 (4)) showing the overlapping leaf bases, each constituting a separate reservoir from which the water has been poured. Dead and half decayed leaves are seen clearly in several places.

Picado (1913) first appreciated the extent of the flora and fauna in tank bromeliads. This fauna is not entirely aquatic: there is a large group of species inhabiting the accumulated humus which stands out of the water. In this connection Picado distinguishes within the whole bromeliad milieu an "aquarium" and a "terrarium" with distinct associated faunas.

The nutrient supply of the tank bromeliad is thus almost exclusively organic. The utilisation of this has not been adequately studied. Picado claims to have demonstrated the secretion of a gum with enzymic properties but there is no evidence of this in the Trinidadian bromeliads. There is, however, an extremely rich microflora in the tank and no reason to doubt its ability to break down the organic material. Picado also demonstrated the ability of a tank species in Costa Rica to absorb amino acids from the interfoliar water.

Many species that are to be grouped under Type III are wholly restricted to the tank for their nutrients. Among the Trinidadian bromeliads *Guzmania lingulata* is such a species. The only humus that accumulates does so within the interfoliar niches of the single leaf rosette which constitutes the entire plant. Other species, however, such as *Gravisia aquilega* (fig. 3 (2)) propagate vegetatively with great vigor and the mature plant comprises a great number of leaf rosettes which not only accumulate humus within themselves but also between themselves. The humus that accumulates between rosettes (and is therefore outside of the "tank") is exploited by the secondary development of functional roots which, as in *Bromelia humilis*, are mycotrophic although to a less extent. This is the habit

of "nest-epiphytism" (Schimper) and table 3 distinguishes as "tank-nest" those species which when fully developed supplement the tank habit with the nest condition.

Ule (1901) has drawn attention to a remarkable association that exists between some ants (*Azteca* and *Camponotus*) and various epiphytes including gesneriads, aroids and bromeliads. The term ant-garden has been applied to this association: indeed, Ule maintained that the epiphytes were planted as seeds by the ants in their nests. This conclusion has been criticized by Wheeler (1921) and Weber (1943), but there is no doubt that the ants at least do not molest the plants. Ant-gardens of this type are very common in Trinidad and two bromeliad species (*Aechmea Mertensii* and *Araeococcus micranthus*) have not been seen by the writer except in such gardens. Both species are of the tank type but have very weak water retaining powers. In both species there is a functional root system penetrating the ant nests and on which it would seem that they are dependent in some way for nutrient. They are classed as myrmecophiles in table 3.

Several bromeliads (*Wittmackia lingulata, Tillandsia bulbosa,* and *T. flexuosa* (figs. 4 and 5)) are so regularly associated with ants (but not ant-gardens) that they should also be regarded as myrmecophilous. In these species ants nest between the leaves which again retain little or no water. It seems likely that the ants' excreta and the material of their nests in some way offset the lack of nitrogenous detritus which is incurred by the poor development of the tank.

Several Type III species—tank bromeliads—have only been found as epiphytes but a considerable number, e.g. *Gravisia aquilega,* may be facultatively either epiphytic or saxicolous. *Vriesia glutinosa* is an obligate saxicole on exposed limestone outcrops in the wetter parts of the Trinidadian mountains.

Type IV: Atmosphere-Absorbing Trichome

The fourth ecological type of bromeliad has been extensively discussed by Mez (1904) in a paper on their water economy. He describes them as "extreme atmospherics." Apart from *Vriesia Platzmannii, V. oligantha, Thecophyllum Kraenzlinianum,* and *Aechmea tillandsioides,* none of which occur in Trinidad, they are all species of the genus *Tillandsia.* The tank habit is lacking entirely and so is any form of absorptive root system. Indeed in *Tillandsia usneoides* the entire root system is lacking. Water is obtained directly from the atmosphere either as dew or rain which is absorbed by the very large *trichompompe* that cover the entire shoot system. It is these large absorbing scales on the leaf blade which give the majority of *Tillandsia* species their characteristic silvery gray appearance (fig. 5).

The nutrient supply of Type IV bromeliads is a long standing puzzle. It is presumably no more than is blown on them in the form of dust. It may be significant in this connection that we observed that several species of Type IV in Trinidad develop nodules on some of their roots before these become fully sclerized. The possibility of these nodules being associated with nitrogen-fixing micro-organisms has not as yet been investigated, but such an eventuality would certainly be in conformity with (a) the total absence of an absorptive root system that could utilise humus on the branch on which the plant grows, and (b) the absence of a tank impounding humus to be exploited by the epidermal absorbing scale. Dubois (1925) reported from greenhouse material an association between *Tillandsia dianthoidea* and the mold *Volutella* which he regards as symbiotic, the fungus providing the bromeliad with nitrogen. *Tillandsia Gardneri* (fig. 5 (1)) is one of the Trinidadian Type IV species. It seems to meet this nutritive problem in a remarkable fashion by curling its leaves around the branch that sup-

ports it, so that the absorbing scales of the leaf blade are directly apposed to the humus on the surface of the branch.

There are many *Tillandsia* species which are essentially intermediate between the "extreme atmospherics" and the true tanks. These intermediates have their leaves arranged in a rosette and their bases covered with absorbing scale and expanded to impound water. These features are typical of Type III. But they also bear on the leaf blades a heavy mat of large absorbing scale which is the hallmark of Type IV. *Tillandsia fasciculata* and *T. utriculata* are such species in the Trinidadian flora.

5. Vertical and Geographic Distribution in Forests and Cacao Plantations

(a) *Relevant Ecological Factors*

Several writers such as Schimper (1888) and Davis and Richards (1933–34) have recognized that epiphytes constitute a more or less independent vegetational unit organized into definite communities. Thus, Schimper recognized as a distinct plant community, a group of epiphytes that can be found both on the low bushes of savannah and the uppermost (exposed) levels in wet forest. Davis and Richards have even spoken of "climax societies" among the epiphytes of the Moraballi Creek forests in British Guiana. In this way they imply some degree of successional development within the communities. There is no doubt that this view is literally correct but the nature of the succession is extremely simple. It is the writer's opinion that truly secondary elements in epiphytic communities are in the minority and in the forest studied in Trinidad (see below) two gesneriads, the *Peperomia* species, and two *Anthuriums* (probably *Anthurium gracile* and *A. scandens*) were the only forms that seemed dependent on the pioneering activity of other epiphytes. Certainly, as Davis and Richards themselves noted in the Moraballi Creek forests, all of the bromeliads (and probably all the orchids) are pioneers and as such are completely independent of other epiphytes.

The only important biotic factors in the ecology of the epiphytic bromeliads are the fauna and microflora of their tanks. Their complete nutritional independence from their substratum which is conferred on them by the tank habit also eliminates any "edaphic" factor. Their relationship with the terrestrial vegetation that supports them is overwhelmingly with (1) its climate and (2) to a less extent with what may be called its mechanical features.

Wherever a particular internal climate is developed in vegetation a particular epiphytic society is likely to develop, almost completely independently of the floristic composition of the supporting terrestrial vegetation. Schimper's case of the identity of some savannah communities with that in forest canopies is a clear example. It is also noteworthy in this connection that in the mountains of northwest Trinidad the community of epiphytes (orchids, bromeliads, and aroids) which occupies the uppermost forest levels successfully occupies bare rocks in well lit openings on the forest floor created by landslides.

The "mechanical" features of terrestrial vegetation that may be considered important ecological factors for epiphytes in general are (a) bark texture, (b) frequency of forks and (c) the angle of limb orientation. In the studies reported here no tendency was observed for individual bromeliad species to occur non-randomly on particular tree species. However, there are many clear cases of particular tree species supporting either a very much heavier or poorer epiphytic growth in general than neighbouring trees of comparable age. Such differences between trees are no doubt due in part to the mechanical features listed above. The Calabash (*Crescentia Cujete*) and all the Myrtaceae are cases in point: the former is undoubtedly predisposed to heavy growths by virtue of its soft deep bark which affords easy anchorage and recess for humus accumulation, while the latter by virtue of their bark shedding habit are always free of epiphytes. However, apart from extreme cases the effect is difficult to detect and usually the differences between trees can far more readily be interpreted on the basis of differences in the microclimate of their canopies which is very largely controlled by the nature and density of foliation. In Trinidad the non-deciduous macrophyllous trees (e.g. *Pachira insignis*) are always freer of epiphytes than smaller-leafed types; and the heaviest growths are usually on microphyllous types whose canopies are well lit.

The fact that the microclimate of the canopy is in general far more important than any mechanical feature is well demonstrated by the effect of climbing vines whose own dense foliage lies on the very top of the tree canopy on which it is climbing. *Carapa guianensis* is a tree that supports a very heavy growth of epiphytes when mature. In the forest strips that were mapped in detail for the bromeliad distributions given later in this paper, it was found that on the same individual *C. guianensis* adjacent limbs could be either covered with epiphytes or entirely free of them. This variation was correlated with the presence or ab-

sence, on the very top of the tree's crown, of a heavy vine growth which rendered the spaces in the canopy immediately below very dark.

The following analysis of the distribution of bromeliad populations in forest is therefore undertaken on the assumption that the factors involved are at least primarily climatic in nature.

(*b*) *The effect of light: Abundance and Vertical Distribution in Forest and Plantation*

The following discussion is limited to a qualitative discrimination of the relative roles of light intensity and humidity as factors affecting bromeliad distributions. A comparison is made of the epiphytic floras from two series of vegetation profiles. The first of these series consists of three profiles which differ maximally in light intensity and minimally in humidity. The second offers the converse conditions: minimal variation in light and maximal variation in humidity.

In this section the data from the first series, concerning the effect of light intensity, are considered. The data consist of the distribution of the bromeliads on two forest strips and one cacao plantation that were all within three hundred yards of each other in Tamana. The first forest strip was far removed from any break in the canopy and was heavily covered with vines in the extreme canopy which severely reduced the light intensity. The second was not only free of vines but stood in the right-angled bend of a river so that it was strongly illuminated both vertically and horizontally. The two strips, although identical in structure and floristic composition, differed markedly therefore in light intensity. They had however very similar humidities. Humidity measurements were made twice at ground level in the two forests when values 96 per cent R.H. and 94 per cent R.H. were obtained in each strip (table 4). The greater exposure of the second strip was evidently completely offset by the immediate proximity of the river. The two strips were two hundred feet long and twenty feet wide. A ground plan was made of all the trees in the strip and this was followed by preliminary drawings of each tree to show the orientation of major limbs. The trees were then felled one at a time and the epiphytic flora mapped in detail. Completed maps of the separate trees were constructed adjusting limb orientations to those shown by the preliminary drawings. Using the ground plan, the finished drawings were then assembled into a profile of the entire strip.

TABLE 4. *Relative humidity measurements at ground level in the two forest strips and one cacao plantation strip whose bromeliad flora is enumerated in table 5.*

	Forest 1	Forest 2	Plantation
First Sample (9. VII. 45)	96%	96%	90%
Second Sample (11. VII. 45)	94%	94%	86%

A sample of cacao plantation of identical size (two hundred feet by twenty feet) includes eight *Erythrina* shade trees which alone support a bromeliad flora: the cacao trees are regularly weeded. The eight *Erythrinas* were drawn to scale, a fair degree of accuracy being obtained by the use of a forty foot extension rod marked off in five foot intervals. All the bromeliads present were then plotted onto the drawings of the separate trees. In plantations the wide planting of the *Erythrinas* and the sparse foliation of their limbs render all the bromeliads visible and identifiable from the ground. The plantation is of course very much more heavily illuminated than the forests but it is also somewhat drier. Humidity measurements at ground level in the plantation made at the same time as the measurements in the forest strips gave values 6–8 per cent lower than in the two forest strips (table 4).

Figure 6 plots the entire bromeliad growth on all three strips without distinguishing species. Two points are evident. First, there is a well-marked overall increase in the flora in the light forest (Forest 2) as against the dark (Forest

1), and a still further increase in the Plantation over Forest 2. Secondly, it is clear that in Forest 2 where the flora is large enough to discern a definite trend in vertical distribution, it is on the whole concentrated in the upper forest levels. At any rate this is so for that part of the strip that is not immediately abutting the river bank. At the left end which overhangs the river there is both a general increase in density of plants and a downwards shift of the vertical distribution. All of these results imply a positive correlation of light intensity and bromeliad abundance. Thus (1) the density of bromeliads increases with increasing light intensity through the three strips, and (2) the density is highest in the forest in the upper well lit levels. The concentration of plants at lower levels in the trees standing on the river bank also correlates with high light intensity and also with high humidity. This heavy concentration of plants in the high humidity of the river bank renders it very unlikely that the fall in humidity (table 4) from Forest 2 to the Plantation is responsible for the increased density in the Plantation.

When the distribution of individual species is considered, striking differences are found between them and it becomes evident that the gross correlation of abundance and vertical distribution with light intensity does not apply equally to the entire flora. For convenience of handling and analyzing these specific distributions, they are placed into one of three groups which are distinguished by their vertical distribution: the groups are stratified one above the other. It must be stressed however that there are no absolute breaks between the distributions of the three groups, each of which contain species that show marked differences between themselves.

Table 5 gives the grouping of the spe-

TABLE 5. *The bromeliad flora on two forest strips differing in light intensity, and an adjacent cacao plantation.* See text for detail. Cf. table 4.

	Forest 1	Forest 2	Plantation
	Dark due to lianes	Well-lit by river at side	Strongest illumination also drier.
EXPOSURE GROUP			
Catopsis sessiliflora	0	4	49
Catopsis floribunda	0	8	3
Vriesia procera	8	28	144
Vriesia amazonica	3	5	5
SUN GROUP			
(i) *Tillandsia fasciculata*	0	1	61
(i) *Guzmania monostachia*	8	60	101
(i) *Aechmea Mertensii*	1	5	0
(ii) *Tillandsia bulbosa*	8	10	2
(ii) *Gravisia aquilega*	0	28	142
(ii) *Aechmea nudicaulis*	6	79	201
(iii) *Guzmania sanguinea*	0	1	0
(iii) *Hohenbergia stellata*	0	4	0
SHADE-TOLERANT GROUP			
Tillandsia anceps	4	34	0
Tillandsia monadelpha	1	26	0
Vriesia albiflora	0	1	0
Vriesia simplex	1	10	0
Guzmania lingulata	48	78	0

cies and their densities in the three strips; figure 7 shows their actual distribution.

The *Exposure Group* occurs in the highest levels of both forest samples. The species it contains occur only under complete exposure to the sky and therefore are concentrated on the crowns of the emergent and semi-emergent trees. In Forest 1 (dark) its members are only present on two extremely microphyllous trees (*Pentaclethra macroloba*) both of which were free of lianes. In Forest 2 it shows a marked general increase still retaining its extreme vertical distribution. However this increase is by no means as great as that which occurs in the plantation. Here, on the widespread and completely exposed *Erythrina* limbs the group is 18 times as dense as in Forest 1. *Vriesia procera* and *Catopsis sessiliflora* are the two species really responsible for this increase.

All the members of the Exposure Group on the two forest strips discussed here are tank species (Type III, table 3) with no tendency to the true nest condition (Type IIIb) which involves the accumulation of humus between the leaf rosettes and the secondary development of roots to exploit this. It will be seen later that in the drier parts of the island the Exposure Group becomes predominantly atmospheric (Type IV) in character.

The *Sun Group* is by far the largest in number of species and is generally distributed throughout the upper canopy levels. It is not only the largest in number of species and individuals but it is the most heterogeneous in that its species show considerable differences among themselves in their vertical distribution. Table 5 distinguishes three subgroups which are again based on different trends in vertical distribution. The two upper subgroups, comprising the species *Tillandsia fasciculata* through *Aechmea nudicaulis* in table 5, show the same pattern of increase from Forest 1 through Forest 2 to the Plantation that obtains for the Exposure species. Here, however, the increase is still more marked.

The Sun bromeliads in the forest are exclusively of the tank type, and although there are several that are purely tank in character (IIIa in table 3), it is in the sun group that the nest habit (IIIb) is fully developed. The nest habit is absent in the Shade-Tolerant group of species (see below) and, as already seen, in the Exposure group. Moreover it is in the Sun group that the largest species, holding the most interfoliar water occur.

The *Shade-Tolerant Group* and the lowest of the Sun group segregates show a very different distribution among the three profiles. All the species occur predominantly in the lowermost levels of the forest canopy, and the Shade-Tolerant species even penetrate the heavily shaded levels near the ground. They all show the same increase in density in the better illuminated Forest 2 over Forest 1, that was found for the Exposure and Sun species. However they are all completely eliminated in the plantation, and the increase ($\times$ 2.9) in Forest 2 over Forest 1 is the lowest of all the groups (table 5). They are all tank species and for the most part what is described as "ephemeral" tanks (Type IIIc) in table 3: only very small amounts of water are retained between the leaves and this often drains away altogether. *Guzmania lingulata* is the only common member of the Shade-Tolerant forms that regularly retains some water but even in this species it is commonly not more than 5 cc. although the plant can hold up to 100 cc.

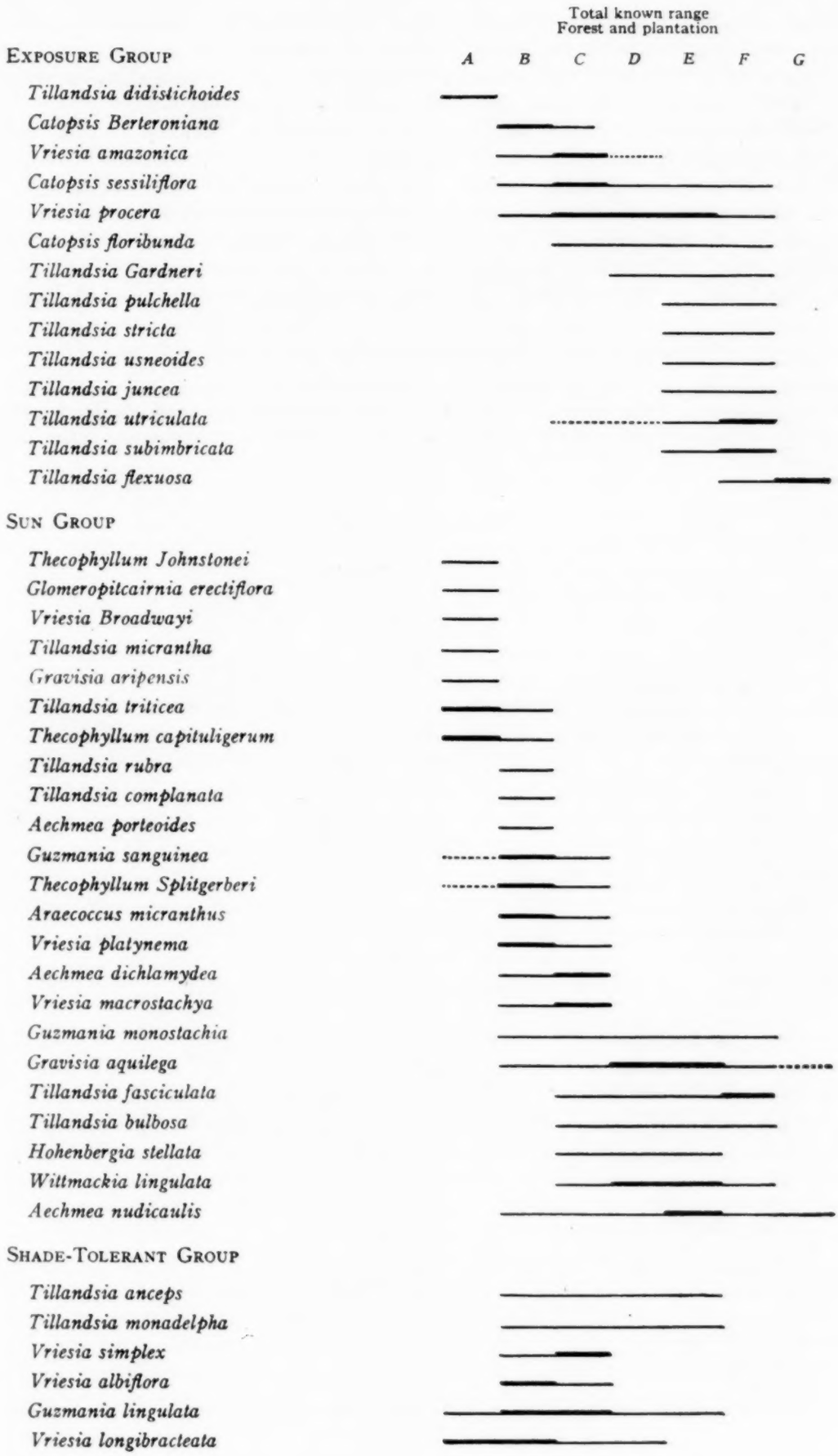
Before discussing further the significance of the details in these distributions, the second series of vegetation profiles has to be considered. This gives data on variation in abundance and vertical distribution of the bromeliads under maximally different humidity conditions and constant light.

(*c*) *The Effect of Humidity: The Bromeliad Flora in Different Rainfall Zones*

The data for this section come from a series of quantitative samples of the bromeliad flora in cacao plantations in a

TABLE 6. *The geographic distribution (in rainfall zones) of the epiphytic Bromeliaceae in Trinidad*

The rainfall zones are indicated by the letters A through G. The heavy line indicates the zone of maximum abundance. Dotted line means very uncommon.



wide range of rainfall zones. Plantations exist in all the rainfall areas in the island, from well over 125″ of rain down to 50″. In all these areas the same species of *Erythrina* is used as "shade" in the plantations and they are laid out with the same 25 ft. × 25 ft. spacing. We have, therefore, a constant forest structure and hence, to all intents and purposes, a constant light intensity under widely varying humidity conditions. This situation is very well suited for distinguishing the effect of humidity, as against light, on the abundance and vertical distribution of the epiphytic bromeliad flora.

The difference between forest and plantation floras in different rainfall zones also yields significant information.

Table 6 lists all the species encountered in the present studies. The bromeliads are listed under the three groups (Exposure, Sun, and Shade-Tolerant) which were

TABLE 7. *The bromeliad flora of cacao plantations in the rainfall zones B, C, D, E, F*

Numerical entries are means (to the nearest whole number) of observed frequencies per five trees on *Erythrina micropteryx*. The statistical variance of these frequencies is treated in table 8. An asterisk indicates that the species concerned does occur in the zone indicated, in spite of the fact that the samples taken for this table failed to include it.

	B	C	D	E	F
Number of samples (each of five trees)	1	12	6	10	6
Bromeliad Species					
EXPOSURE GROUP					
Catopsis Berteroniana	10	<1	0	0	0
Catopsis sessiliflora	3	8	1	1	<1
Catopsis floribunda	1	6	3	12	1
Vriesia amazonica	1	8	0	<1	0
Vriesia procera	32	62	49	55	24
(*Tillandsia Gardneri*)	0	0	0	*	*
(*Tillandsia pulchella*)	0	0	0	*	*
(*Tillandsia stricta*)	0	0	0	*	*
(*Tillandsia usneoides*)	0	0	0	*	*
(*Tillandsia juncea*)	0	0	0	*	*
Tillandsia utriculata	0	0	0	6	17
Tillandsia subimbricata	0	0	0	4	42
Tillandsia flexuosa	0	0	0	0	1
SUN GROUP					
Tillandsia rubra	5	0	0	0	0
Tillandsia complanata	3	0	0	0	0
Aechmea porteoides	97	0	0	0	0
Guzmania sanguinea	50	0	0	0	0
Thecophyllum Splitgerberi	*	<1	0	0	0
Vriesia platynema	63	1	0	0	0
Vriesia macrostachya	0	2	0	0	0
Aechmea dichlamydea	0	*	0	0	0
Guzmania monostachia	322	54	79	74	56
Gravisia aquilega	*	67	94	78	38
Tillandsia fasciculata	0	35	71	60	119
Tillandsia bulbosa	0	2	3	5	0
Wittmackia lingulata	0	13	140	67	8
Aechmea nudicaulis	55	79	189	415	89
SHADE-TOLERANT GROUP					
Guzmania lingulata	52	<1	0	0	0
Tillandsia anceps	<1	<1	0	0	0

TABLE 8. *The density of bromeliads per five trees of* Erythrina micropteryx *in the cacao plantations of the four rainfall zones C, D, E, and F*

The values given are means (and their standard errors) of square root transformations of actual frequencies per five trees.

Number of samples (of five trees)	C 12	D 6	E 10	F 6	P values of indicated density changes
Catopsis sessiliflora	2.1±0.5	0.5±0.5	0.6±0.4	0.2±0.2	C to F; P<0.05
Vriesia amazonica	2.5±0.5	0.0	0.1±0.1	0.0	C to D, E, F; P negligible
Vriesia procera	7.8±0.7	6.2±1.6	7.3±0.8	3.7±1.8	No significant change
Guzmania monostachia	6.9±0.8	8.8±0.5	8.5±0.4	7.1±1.3	No significant change
Gravisia aquilega	8.0±0.7	9.3±1.0	8.5±1.0	5.0±2.2	No significant change
Wittmackia lingulata	(1.5±1.0)	8.9±4.3	6.9±1.7	1.2±1.2	E to F; P<0.05
Aechmea nudicaulis	8.3±0.8	13.0±2.3	20.1±2.2	8.3±2.0	E to C or F; P<0.02
Tillandsia fasciculata	5.6±0.7	8.2±1.2	7.5±0.7	10.6±1.3	C to F; P<0.05
Tillandsia utriculata	0.0	0.0	2.1±0.5	3.9±0.8	C & D to E or F; P negligible
Tillandsia subimbricata	0.0	0.0	1.3±0.6	6.4±0.5	C & D to E or F; P negligible

recognized in the previous section. The table indicates the total range of the various species in the seven climate zones (A through G) in the island so far as determined by us in three years' observations; zones of maximum abundance are indicated by the heavier line. This range includes either forest or cacao plantation or both and may be compared with the range for plantation alone given in table 7. The data in table 7 were obtained from the enumeration of the flora on sample strips of five *Erythrinas* in cacao plantations. As indicated in the table several samples (of five trees) were taken in each rainfall zone and the tabulated plant densities are means from these samples.

It is clear from tables 6 and 7 that the specific composition of the bromeliad flora, and the relative abundance of the various species, changes markedly with change in rainfall level. The cloud forests of the northern mountain peaks (Zone A) have a flora of six bromeliad species that are wholly restricted to them. The wet mountain slopes and valleys (Zone B) below the peaks have an almost equally distinctive flora which does, however, merge somewhat with that of the eastern foothills of the northern range and the wet eastern slope of Mount Tamana (Zone C). From Zone C through Zone F the specific composition of the flora undergoes much less change but there are marked shifts in the relative densities of some of the species concerned.

The discussion in this section is restricted to these changes from Zone C through Zone F since it is for this section of the gradient that quantitative data on both abundance and vertical distribution in plantations are available.

In order to investigate the significance of the differences in bromeliad frequency between different zones, an analysis was made of the square roots of the numbers found in the five-tree samples. The square root was used since inspection of the raw data showed the variability of the original numbers to be greater, the greater their mean: such a transformation has proved useful in similar situations. The means of the square roots and their standard deviations are given in table 8 along with probabilities (P values) for the observed changes in density.

The principal changes within the C to F gradient are as follows. (1) Three species, *Gravisia aquilega*, *Guzmania monostachia*, and *Vriesia procera*, are abundant throughout the whole range of rainfall levels and they show no (statistically) significant change in abundance; although it is believed that the slight drop in F shown by both the *Vriesia procera* and *Gravisia aquilega* samples reflects a

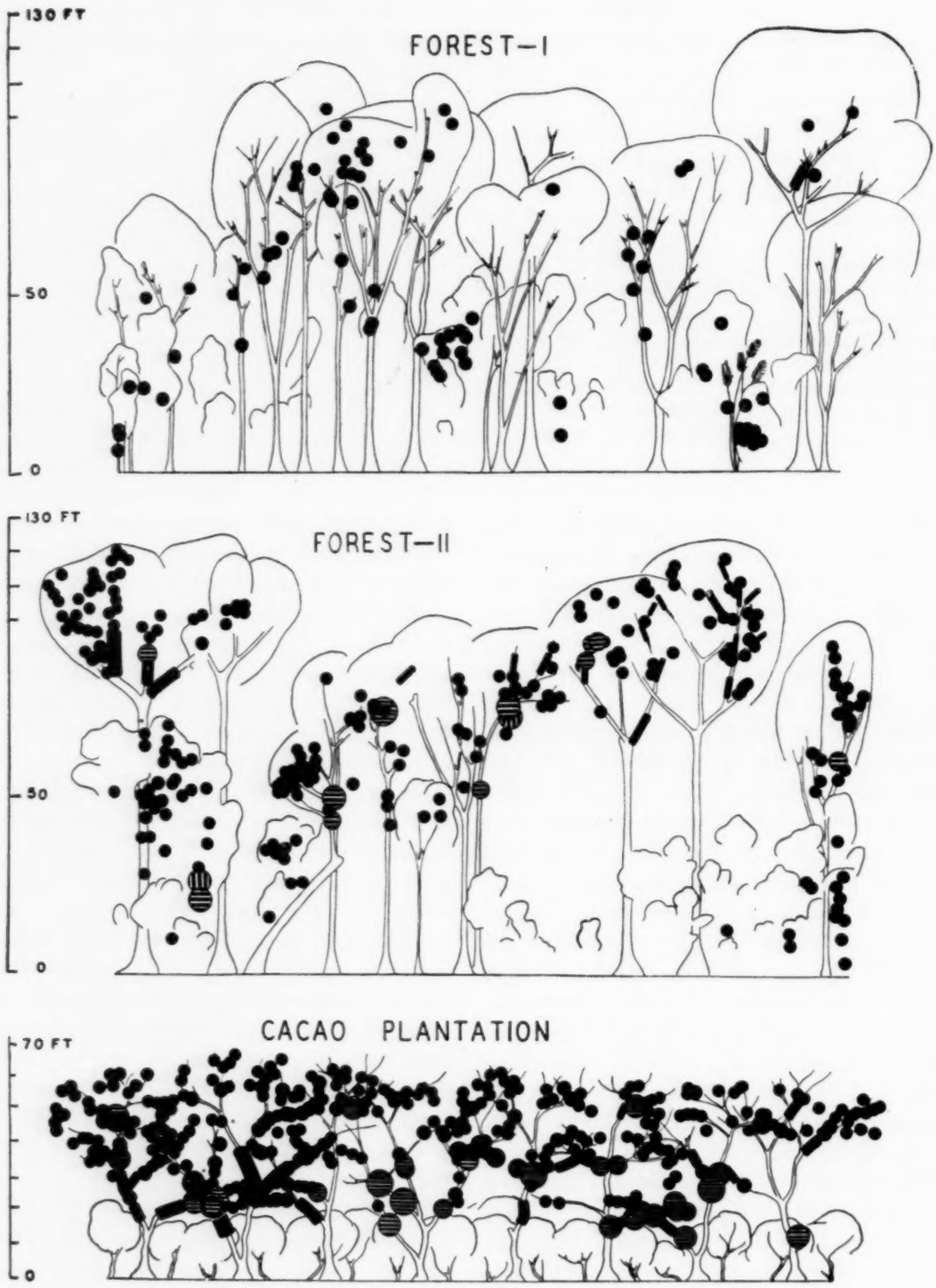


FIG. 6. The entire bromeliad flora on two forests differing only in light intensity, and an adjacent cacao plantation. Only *Gravisia aquilega, Hohenbergia stellata,* and *Aechmea nudicaulis* are distinguished on this figure by the symbols used in figure 7. All other species indicated simply by a black circle. See text for detail.

real decline of the actual population of these two species in the F Zone. (2) The large tank species of the genus *Vriesia* in the Exposure (*V. amazonica*) and upper Sun groups (*V. platynema* and *V. macrostachya*) are restricted to the areas of highest humidity. They decline in abundance very sharply below the C Zone. (3) *Wittmackia lingulata,* a markedly xeromorphic nest-tank type of the Sun group, increases to a very well defined peak in the drier sections of Zone D. (4) This is true also for *Aechmea nudicaulis* which has, however, its maximum in Zone E. *A. nudicaulis,* a tank species, is also very strongly xeromorphic. (5) *Tillandsia fasciculata,* an intermediate atmospheric (Type IVa, Table 3), shows a steady increase from C to a maximum in F. *Tillandsia subimbricata* and *T. utriculata* are even more restricted to the drier end of the gradient (Zones E and F). (6) The great majority of *Tillandsia* species in the island belong in the Exposure group. They are limited, like the three *Tillandsia* species just described, to the driest sections of the island. They occur only in the 50″ – 90″ rainfall areas (Zones E – F) in the northwest of the island. (The E and F samples for table 7 were taken from the west central part of the island where several of the *Tillandsia* species like *Gardneri, pulchella,* and *juncea* are absent.)

Table 9 gives the vertical distribution of the bromeliads in specimen samples from the C, D, and F rainfall zones. The data for each zone include two five-tree samples. The table illustrates four points.

(1) There are considerable and constant differences between the species in their vertical distributions. *Vriesia procera, Catopsis sessiliflora,* and *Vriesia amazonica* have the highest distributions.

TABLE 9. *The vertical distribution of bromeliads in cacao plantations in the zones C, D, and F*
The data for each zone are the total bromeliads from two 5-tree samples. See text for detail.

		Height intervals in feet							
		0–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80
Zone (C)	*Vriesia procera*	0	0	0	6	11	32	32	17
	Catopsis sessiliflora	0	0	0	0	0	5	5	0
	Vriesia amazonica	0	0	0	4	6	10	6	0
	Tillandsia fasciculata	0	1	7	12	40	16	13	1
	Guzmania monostachia	0	4	3	23	25	21	13	2
	Gravisia aquilega	0	3	32	20	50	47	0	0
	Aechmea nudicaulis	0	30	36	25	34	12	6	0
	Wittmackia lingulata	0	0	0	0	0	0	0	0
Zone (D)	*Vriesia procera*	0	5	37	50	50			
	Catopsis sessiliflora	0	0	1	3	6			
	Vriesia amazonica	0	0	0	0	0			
	Tillandsia fasciculata	4	24	71	85	47			
	Guzmania monostachia	0	22	26	30	11			
	Gravisia aquilega	5	59	35	16	4			
	Aechmea nudicaulis	3	54	105	46	13			
	Wittmackia lingulata	0	50	0	0	0			
Zone (F)	*Vriesia procera*	0	3	10	34	36	40		
	Catopsis sessiliflora	0	0	0	0	0	0		
	Vriesia amazonica	0	0	0	0	0	0		
	Tillandsia fasciculata	0	5	44	82	149	74		
	Guzmania monostachia	0	19	61	61	39	34		
	Gravisia aquilega	0	1	18	1	18	41		
	Aechmea nudicaulis	0	0	61	4	48	2		
	Wittmackia lingulata	0	0	0	0	0	0		

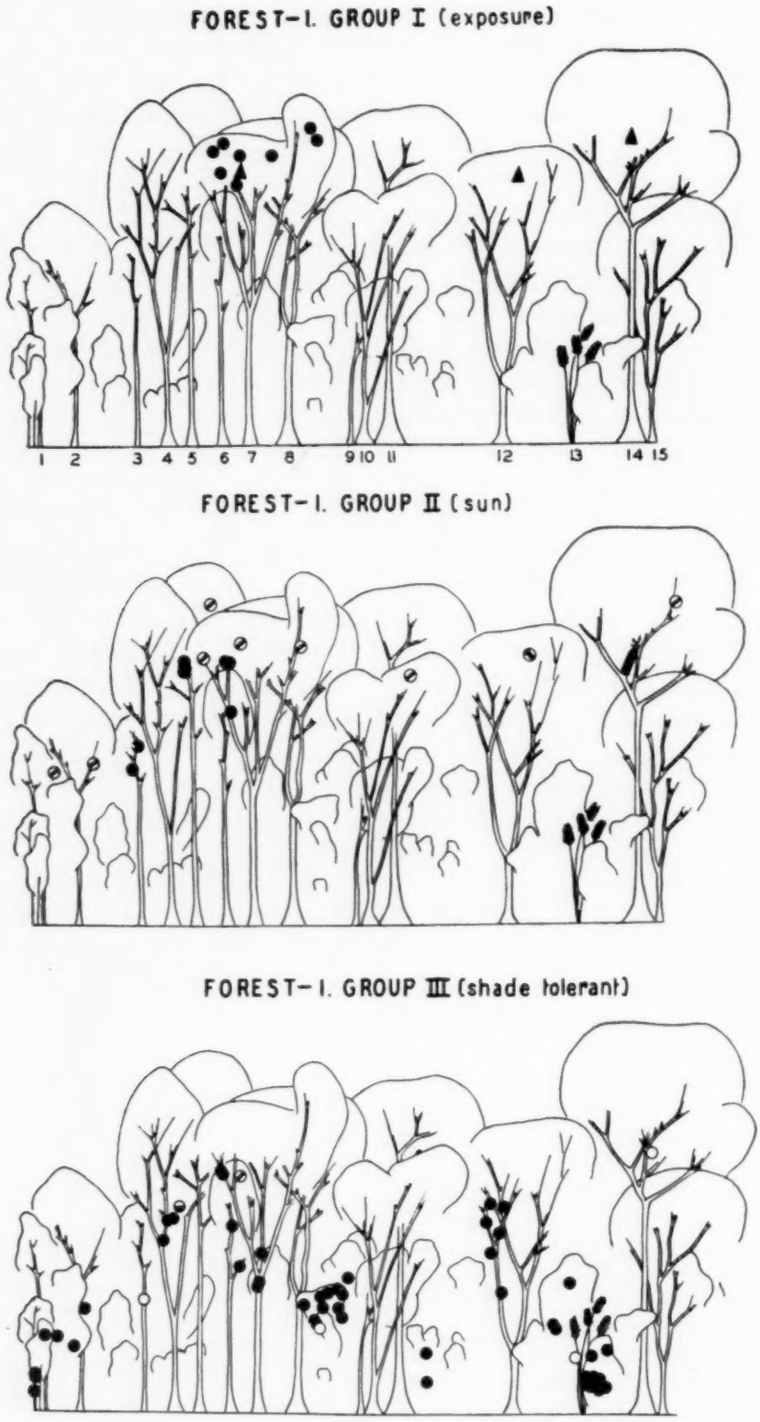


FIG. 7a

FIG. 7. The abundance and vertical distribution of the bromeliads on (a) Forest 1, (b) Forest 2, and (c) the Cacao Plantation. See text.

Three groups of bromeliads, Exposure, Sun, and Shade-Tolerant, are recognized in each vegetation sample. Individual species are distinguished by symbols that are given in 7c under the appropriate group within which they apply; e.g. the solid black circle means *Guzmania lingulata* in the Shade-Tolerant group on all three profiles (Forest 1, Forest 2, and Plantation),

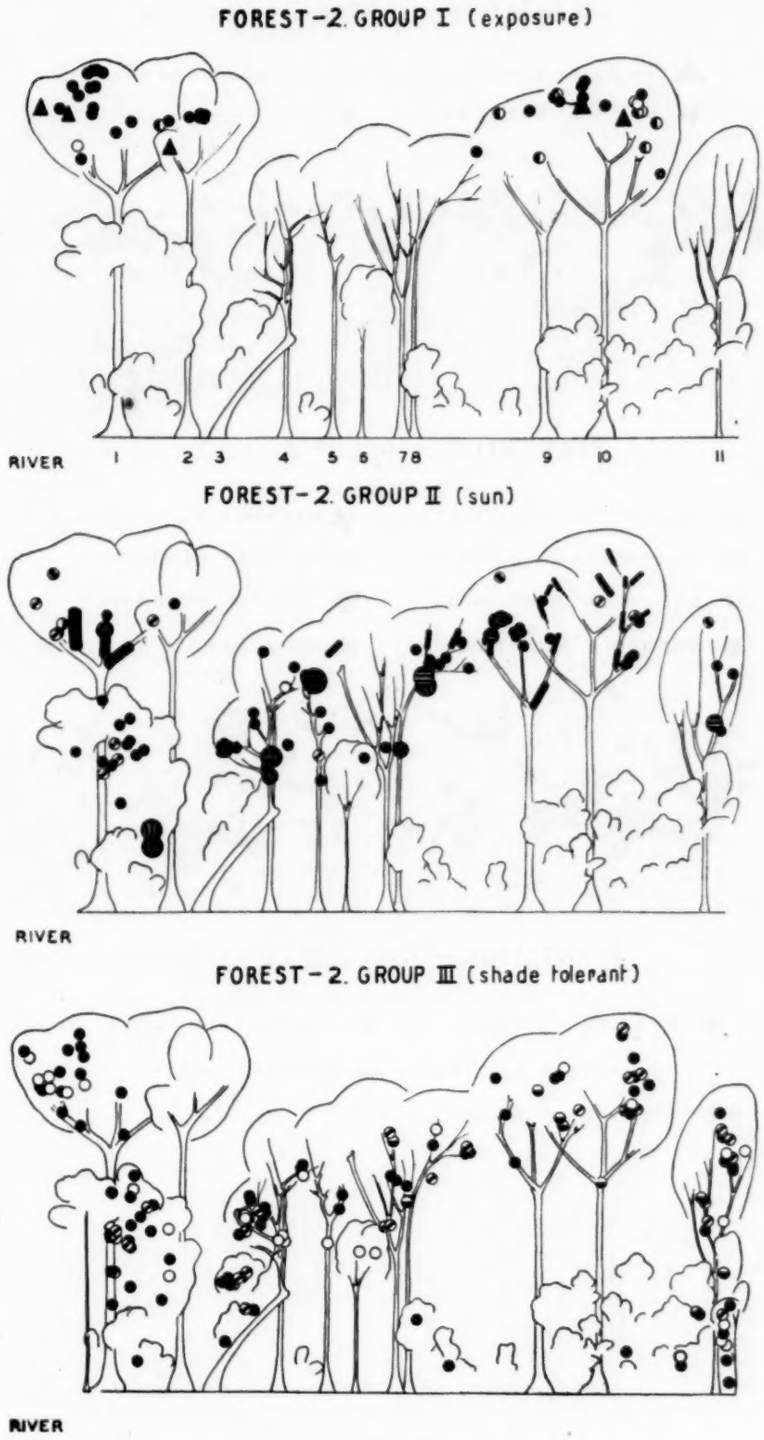


FIG. 7b

but it means *Guzmania monostachia* in the three Sun group profiles, and *Vriesia procera* in the Exposure group profiles.

For all species one symbol equals one plant. *Gravisia aquilega*, *Aechmea nudicaulis*, and *Hohenbergia stellata* propagate vegetatively. In these three cases the size of the symbol is proportional to the number of leaf rosettes on the plant.

The numbered trees are identified in the caption of fig. 7c.

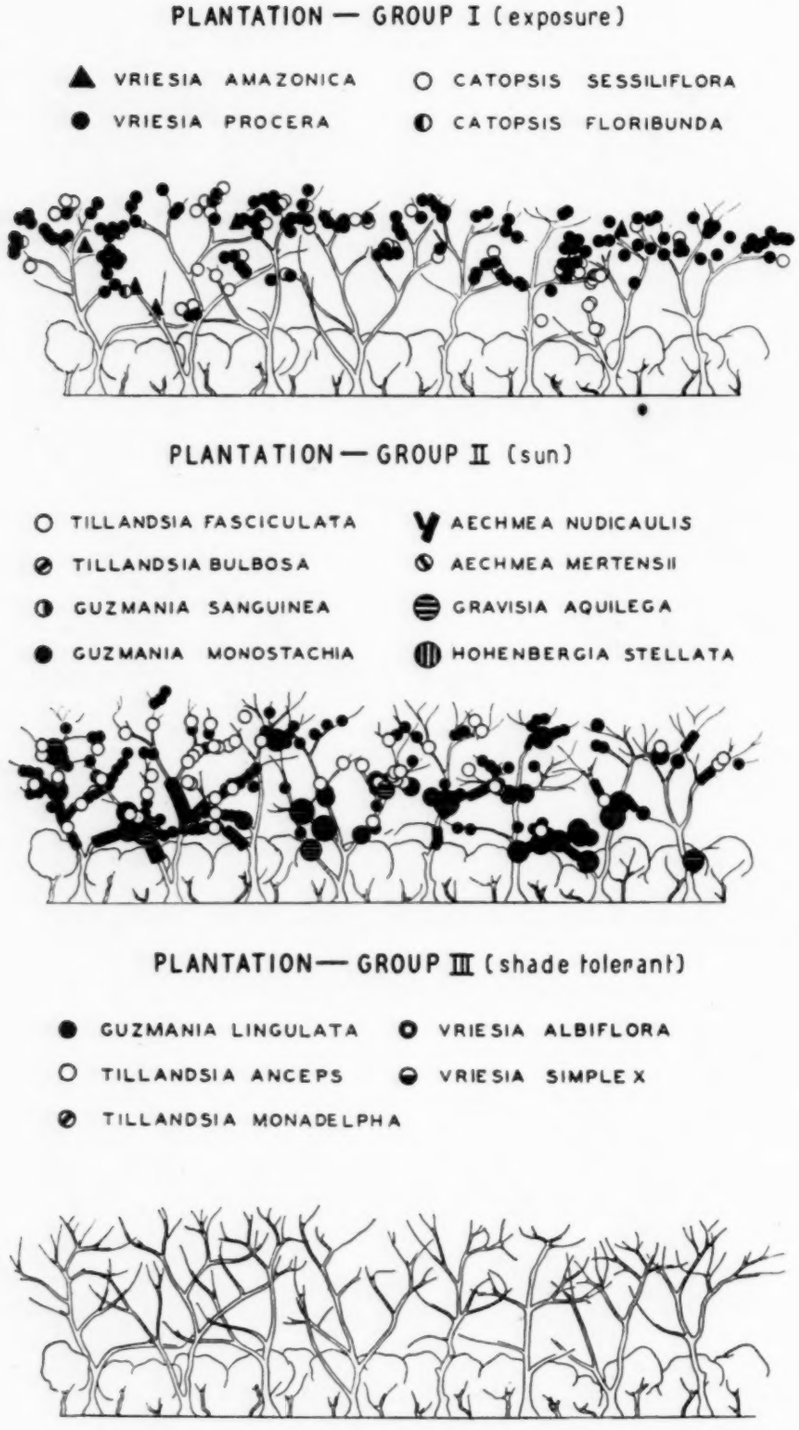


FIG. 7c

The identification of numbered trees is as follows: *Carapa guianensis*, 8, and 14 in Forest 1; 5, 9, and 10 in Forest 2. *Eschweilera subglandulosa*, 11 in Forest 1; 2 in Forest 2. *Sterculia caribaea*, 6 in Forest 1; 1 in Forest 2. *Pachira insignis*, 5, and 9 in Forest 1; 4, 6, and 8 in Forest 2. *Pentaclethra macroloba*, 2, 4, 7, 10, 12, and 15 in Forest 1; 7 and 11 in Forest 2. *Andira inermis*, 3 in Forest 2. *Symphonia globulifera*, 3 in Forest 1. This nomenclature follows that given by Beard (1946).

These are three members of the Exposure group (cf. fig. 7c and table 5). *Tillandsia fasciculata* is regularly lower than *Vriesia procera* and higher than *Guzmania monostachia*. The large tank species, *Gravisia aquilega* and *Aechmea nudicaulis*, have a wider and more irregular distribution on the profile but they occur much lower than the others.

(2) The irregularity of the large species is due in part to the fact that the scored frequencies are of leaf rosettes (i.e. units such as that shown in figure 3 (3)) and not of individual plants. A single plant (zygote) of *Aechmea nudicaulis* may comprise fifty leaf rosettes produced by vegetative propagation. Rosettes were scored not only because they are the significant units as far as mosquito breeding places are concerned but because it is, in practice, impossible to determine whether or not a clump of, say, twenty rosettes is composed of one or more individual plants. The great variation (age?) between individuals in the vigor of their vegetative propagation adds greatly to the variance on the apparent frequency of these species.

(3) The *Erythrina* shade trees in plantations vary in height from just under fifty

TABLE 10. *Summary of data on the vertical distribution of bromeliads in cacao plantations in different rainfall zones*

C = ca. 125″ rain; D = 90″–110″; E = 70″–90″; F = 50″–70″. Samples from the plantations under 60 feet high and over 60 feet high are treated separately. None of the F Zone samples were over 60 feet high. Entries in the table absolute numbers of plants observed.

	Height intervals in feet	Samples over 60 feet high			Samples under 60 feet high			
		drier →			drier →			
		C	D	E	C	D	E	F
No. of 5-tree samples		8	2	6	4	4	2	4
Vriesia procera	50+	325	77	245	131	114	50	86
	30–50	236	4	105	44	32	15	54
	0–30	9	0	9	4	1	0	3
Catopsis sessiliflora	50+	35	0	0	11	0	0	0
	30–50	37	0	4	10	0	0	0
	0–30	0	0	0	0	1	0	0
Vriesia amazonica	50+	19	0	0	56	0	0	0
	30–50	29	0	1	26	0	0	0
	0–30	6	0	0	0	0	0	0
Tillandsia fasciculata	50+	71	63	157	89	82	34	344
	30–50	157	26	176	68	88	23	221
	0–30	40	1	45	1	11	0	13
Guzmania monostachia	50+	136	60	221	80	179	34	93
	30–50	239	39	211	93	156	52	157
	0–30	92	3	68	3	18	1	30
Gravisia aquilega	50+	62	24	86	32	38	10	59
	30–50	309	62	163	117	236	72	19
	0–30	251	17	237	36	86	84	1
Aechmea nudicaulis	50+	78	147	938	50	274	154	74
	30–50	248	178	1395	173	363	496	203
	0–30	344	50	247	40	44	71	37
Wittmackia lingulata	50+	10	41	107	0	18	0	23
	30–50	44	409	173	0	140	21	25
	0–30	101	137	191	0	124	12	0

feet to just over eighty feet. Although neighboring trees, as in the individual five-tree samples, usually have the same approximate height (cf. fig. 7c) this variation is sufficient to raise obvious difficulties in the statistical comparison of the vertical distribution of bromeliads in different zones.

(4) Inspection of table 9 shows that the various species show no marked distributional shift on the profile of the plantations in the three zones. For example, although in D and F *Vriesia procera* occurs in the levels below thirty feet from which it is absent in Zone C, this is simply due to the plantations being lower in D and F and does not represent a real shift downwards on the microclimatic gradient within the plantation. The maximum density of the species remains in the highest (most exposed) levels.

Table 10 summarizes the vertical distribution of the species in the thirty samples of 5 trees taken from Zones C through F. It is obvious that no simple mean or lumped distribution can be given for samples that range from fifty to eighty feet without obscuring the real distribution patterns of the various species and minimizing the differences between them. In table 10 this problem has been met in part by treating separately those samples that are less and greater than sixty feet in height.

The summaries in table 10, like the specimen detailed distributions in table 9, give no evidence at all of any systematic shift in vertical distribution correlated with changing rainfall.

The important point is that this applies even to those species whose density is limited by changing rainfall level. *Catopsis sessiliflora* and *Vriesia amazonica* become very rare in all zones drier than C (ca. 125″). The disappearance is an abrupt one and careful field search has failed to detect any systematic trend down the profile on the periphery of their rainfall range. The same pattern applies for a number of other species and is especially striking in moving out of the wet valleys of the northern mountains (B zone). Here *Aechmea porteoides, Guzmania sanguinea, Vriesia platynema* (table 7) are very common, but they disappear sharply in the drier foothills without shifting downwards in plantations.

(*d*) *The Relative Roles of Light and Humidity in Determining Vertical and Geographic Distribution*

A comparison can now be made of the data from the two series of observations just described—in one of which light is the prime variable, and in the other, humidity (rainfall). In considering the effect of these variables on the bromeliads two aspects of distribution—vertical and geographic—will be distinguished.

(1) The tank species of the Exposure and Sun groups will be treated first. Three distinct lines of evidence indicate that light is the major climatic variable for this group of species. First, the species in these two groups showed a steady and striking increase in density in the three profiles (Forest 1, Forest 2, Plantation) of increasing light intensity (fig. 7; table 5). Secondly, they have a characteristically high vertical distribution in the well lit canopy strata of forest, and appear at lower levels only where the canopy breaks and allows light to penetrate, as at the river bank end of Forest 2 in figure 7b. These first two lines of evidence are in themselves strongly indicative that light is the major ecoclimatic variable. Thus, our sampling (table 4) detected no difference in humidity at ground level between Forest 1 and Forest 2, and the possibility that the known humidity drop in the Plantation is primarily responsible for the immense increase in bromeliad density there, is extremely unlikely since the saturated atmosphere at the river edge has no diminishing effect on the flora at the left end of Forest 2.

The third line of evidence is quite unequivocal: there can be no doubt that the distribution of bromeliads in the higher levels of forest vegetation is related primarily to the high light intensities

rather than the low humidities of these levels. If their vertical distribution were determined primarily by humidity, the bromeliads would show shifts in vertical distribution as overall humidity levels changed. In areas of unfavorably low humidity there should be a shift downwards, and the data on the vertical distribution of the Exposure and Sun species in plantations from Zones C to F (tables 7 and 8) give no evidence for this whatsoever, even, as already noted, in those species such as *Vriesia amazonica* whose distribution is completely limited by decreasing rainfall. Both humidity and light each play a part in determining total distributions in the Exposure and Sun tank species, but light is the dominant factor in that there is virtually no latitude in tolerated light intensities whereas the plants range widely through varying rainfall levels. Once the limit of tolerated humidity is passed a species at a given level (light intensity) in plantation disappears rather than adjust its level to accommodate the humidity changes.

(2) A group of species were classified as Shade-Tolerant on the basis of their distributions as given in figure 7. When their geographic and vertical distributions are considered together conclusions are reached which differ, in part quite sharply, from those found for the Exposure and Sun tank species. First of all, there is, however, agreement between the groups in that for both, abundance shows a strong positive correlation with light intensity. This is demonstrated by the considerable increase in the Shade-Tolerant species in the well-lit Forest 2 over Forest 1, and also by the fact that the majority of individuals do occur in the upper half of the forest profile (fig. 7).

However, they differ markedly from the Exposure and Sun species in the following respect: they tolerate a wide range of light intensities to accommodate a primary requirement for high humidity. The data leading to this conclusion come from two sources. First, two of the species concerned in the Tamana profiles (fig. 7), *Guzmania lingulata* and *Tillandsia monadelpha*, occurred not only in the lower levels of Forests 1 and 2 which were the deepest shade sampled, but they were also present on the limbs of the *Sterculia caribaea* which overhangs the river in Forest 2 and is one of the most fully illuminated niches sampled. On the outermost limbs of the *Sterculia*, at least the *Guzmania lingulata* specimens are in as full an insolation as occurs in the extreme forest canopy and the plantation, from both of which situations this species, and all the other shade tolerant forms, are absent. Those situations where the Shade-Tolerant species do occur may differ extremely in light intensity but they are all highly humid.

Secondly, although *Guzmania lingulata*, which is the most abundant member of the group, does not appear in the strong illumination and relatively low humidity of the plantations in Zones C through F, it is abundant in plantations of identical structure in the very humid B Zone where the rainfall is 200″ or more. (See tables 6 and 7.)

Vriesia longibracteata is a member of Shade-Tolerant group with a still higher humidity requirement than *Guzmania lingulata*. It is rare in the Tamana region and unrepresented on the profiles sampled for figure 7. In its distribution pattern it shows the same features as *Guzmania lingulata* only to an exaggerated degree. It is commonly found in intense shade so near the ground on tree buttresses that temporary rain pools partly inundate it. However, its typical habitat in Trinidad is on those fully insolated branches that droop out from the forest to overhang the surface of rivers. Here there is the combination of high light intensity, very high relative humidity, and even occasional inundation. Like *Guzmania lingulata* and the other Shade-Tolerant species, *Vriesia longibracteata* holds a little water between its leaves, a fact that is surely correlated with dependence on high humidity.

(3) Finally, the "atmospherics" (Type

IVb) and "intermediates" (IVa) require separate discussion. Here again the group occurs only in habitats of extremely high light intensity; all but *Tillandsia fasciculata* are placed in the Exposure group and the latter species is a member of the upper subgroup of the Sun species. As with the tank species of the Exposure and Sun groups there is no evidence that the atmospherics or intermediates shift their distribution on the light gradient in plantations to accommodate unfavorable changes in humidity. In this repect light is again to be regarded as the dominant ecoclimatic variable. However, humidity is certainly a more important factor in determining the distribution of Type IV species than it is for the tank (Type III) species.

Mez (1904) has noted in his discussion of the water economy of the atmospheric species that their characteristic feature—the presence of a dense mat of overlapping absorbing scales over the entire leaf blade—has two distinct properties. It enables them to exploit fully all the dew that forms on the leaves by immediately drawing it into the system of fine capillaries created by the overlapping absorbing scales on the epidermis, but in some species at least it also imposes difficulties in connection with respiration. Since the absorbing scales overlie the epidermis, regular desiccation of the capillary system between them is necessary for gaseous exchange through the stomata. Mez regarded this as the reason for the reported complete absence from rain-forest of such otherwise ubiquitous Type IV species as *Tillandsia usneoides* and *T. recurvata*. Thus the elaborate armature of epidermal scales which the atmospherics possess not only permits them to exploit but also limits them to extremely dry habitats.

One species in Trinidad which is of Type IVa, *Tillandsia didistichoides* (fig. 2 (2)), seems to contradict this notion since it occurs only on the highest peaks of the northern mountain range which are virtually constantly in rain. It may be, however, that the high winds which are incessant on these peaks effect desiccation of the leaf surfaces as soon as rain ceases for a moment. Certainly the plants occur only on the remotest, most fully exposed branches of the trees they infest, and they are absent in the more protected forests immediately below the peak.

In any case, *Tillandsia didistichoides* aside, the distribution of the many other Trinidadian atmospherics and intermediates is in marked conformity with the view Mez advanced for the absence of *T. usneoides* and *T. recurvata* from forest. All the Type IV species of *Tillandsia* in Trinidad are either wholly restricted to the driest parts of the island or, in the case *T. fasciculata* and *T. utriculata*, they are most abundant in these regions and decline steadily with increasing rainfall level (tables 6 and 7). As already noted they are all Exposure species, with the exception of *T. fasciculata*, which belongs in the uppermost Sun group.

The detailed distribution of *Tillandsia fasciculata* on the series of two forest profiles and the plantation profile in Tamana is further evidence of the importance of low humidity for the Type IV bromeliads. It is quite abundant (60 plants) on the plantation strip, where it occurs slightly but significantly lower than *Vriesia procera*, a relative position which is maintained throughout all of the available samples (fig. 7c; tables 9 and 10). However, apart from one specimen in Forest 2 it is completely absent from the forest strips adjacent to the plantation where it is so common. Moreover, it is clear that its absence from the forest is not due to inadequately high light intensities, since in the forest there is a substantial population of *Vriesia procera* which, judged on the basis of constant vertical distribution, has a greater light demand than *T. fasciculata*.

Finally, it should be pointed out that it is only *Tillandsia fasciculata* and the Shade-Tolerant group that have a really discontinuous distribution between forest and plantation in the data of figure 7. The remainder, the entire flora of tank

species in the Exposure and Sun groups, shows a steady increase with light intensity in spite of the sharp drop in humidity (table 4) in the cacao. It is noteworthy that for both *T. fasciculata* and the Shade-Tolerant group there are other grounds for inferring a greater sensitivity to humidity conditions than characterizes the Sun and Exposure tank species; the sharp discontinuity of their distribution between forest and plantation is independent evidence for this.

6. Ecological Evidence on the Evolutionary History of the Bromeliaceae

In 1888 A. F. W. Schimper developed the idea that epiphytes in general have evolved from the terrestrial flora within wet forests. He envisaged the high humidity of the forest's atmosphere as the major factor that has permitted the plants to divorce themselves from the usual source of water in the soil.

Tietze (1906) has taken an entirely different view of the origin of the epiphytic Bromeliaceae: he maintained that they have arisen from a stock inhabiting semi-desert conditions. His argument is as follows: (1) On the basis of the comparative morphology of the flower, seed and absorbing scale, the Bromeliaceae is to be regarded as monophyletic; (2) of the three subfamilies (table 11) the Pitcairnioideae is the most primitive, (3) it is almost exclusively xerophytic, and (4) its members possess epidermal trichomes of a low degree of organisation that, judging from their connection with the mesophyll, probably do have a water-absorbing function. (5) The Tillandsioideae and Bromelioideae have evolved from the Pitcairnioideae and their success as epiphytes is due principally to the further elaboration of the epidermal trichomes to become an absorbing system taking in nutrients as well as water.

This theory of a desert rather than a rain forest origin of epiphytism in the Bromeliaceae seems, from the outset, very plausible. However, it is open to criticism so long as it rests on the specific

Table 11. *The subfamilies of the Bromeliaceae (cf. Smith, 1934)*

Subfamilies with Trinidadian genera	Ecological type*	Ovary	Fruit	Seed	Leaf margin	Basic chromosome number***
Pitcairnioideae *Pitcairnia*	I	Superior, to half inferior	Capsular, dehiscent	Winged	Spinose-serrate	$x=25$
Bromelioideae** *Bromelia* *Ananas* *Araeococcus* *Wittmackia* *Hohenbergia* *Canistrum* *Gravisia* *Aechmea*	I, II, III, IV	Wholly inferior	Baccate, indehiscent	Naked	Spinose-serrate	$x=25$ $x=8$ $x=9$ } Rarer
Tillandsioideae *Tillandsia* *Guzmania* *Vriesia* *Thecophyllum* *Catopsis* *Glomeropitcairnia*	III, IV	Wholly superior (except for *Glomeropitcairnia*)	Capsular, dehiscent	Plumose appendaged	Naked	$x=8$

* See table 3. ** *Billbergia* is another bromelioid genus listed by Broadway and Smith (1933) for Trinidad, but is only known there from cultivation. *** Chromosome numbers from Lindschau (1933).

argument—the ancestral position of the Pitcairnioideae—which Tietze developed from his herbarium material.

Table 11 lists the principal features that separate the three subfamilies of the Bromeliaceae. The two higher subfamilies containing the epiphytes are extremely well differentiated from each other. All the Tillandsioideae have a hypogynous corolla, a capsular fruit and plumose appendaged seeds; all the Bromelioideae are epigynous, have baccate fruits and naked seeds. Since there are still many genera in the Bromelioideae which are primitively terrestrial, it is clear that this (baccate) subfamily diverged from the ancestral capsular bromeliads (Smith, 1934) while the latter were also still terrestrial. Epiphytism has therefore evolved quite independently in the Tillandsioideae and the Bromelioideae and the available evidence on the pattern of this evolution must be considered for the two sub-families separately.

The Tillandsioideae. It is true that the Bromeliaceae may be described as monophyletic if this be taken to mean simply that the whole family is derived ultimately from a common ancestral stock. It by no means follows however, as Tietze infers, that the most primitive of the three contemporary subfamilies has given rise directly to the other two. Indeed the evidence is strongly against this. Smith has pointed out that the bulk of the Pitcairnioideae are disqualified as ancestors owing to their half epigynous corollas. This condition certainly cannot be ancestral to the hypogyny of the entire Tillandsioideae. Smith (1934) shows that *Puya*—which is hypogynous—is the only genus with reasonable claims to be considered as really ancestral even to the other Pitcairnioideae. It is still however a long way from having close relationship with the Tillandsioideae since Lindschau's (1933) cytological data show that *Puya*, like all the other pitcairnioids she examined, has the basic chromosome number $x = 25$ ($2n = 50$) while all the tillandsioids have $x = 4$ ($2n = 32, 56, 64$ and 96). The Tillandsioideae stands wholly apart from the rest of the family in other respects besides this cytological difference. The epidermal trichome is not only distinct in its detailed morphology (Tietze, 1906) but throughout the *whole* subfamily it has reached its fullest functional development as an absorbing organ taking in both nutrients and water. All the tillandsioids are, nutritionally, entirely independent of the substratum and there are no traces of the intermediate ecological steps involved in the transition from the primitive terrestrial condition.

It is clear then that the appeal to the xerophytism of the contemporary pitcairnioids is in itself not a cogent argument in favor of the desert origin of the Tillandsioideae. All three subfamilies are more properly regarded as separate derivatives of a common ancestral group which may or may not have been as exclusively xerophytic as the present day Pitcairnioideae. It follows, therefore, that evidence concerning the evolutionary history of the Tillandsioideae is best taken from within the subfamily itself. There are three lines of such evidence available, two of them from the ecological data presented in this paper, and one from the physiology of the trichompomp (absorbing scale) as Mez (1904) describes it.

(1) A comparison of the ecology of those genera that, on taxonomic grounds, are considered "younger" with those that are "older" gives direct evidence that the Tillandsioideae have not arisen from a

TABLE 12. *The number of species in the six Tillandsioid genera in the climatic (rainfall) zones in Trinidad.* See Table 6; also page 61 for details of the climatic zones.

	Drier →						
	A	B	C	D	E	F	G
Tillandsia	3	5	5	6	11	10	1
Guzmania	1	3	3	2	2	2	0
Vriesia	2	7	7	3	1	1	0
Thecophyllum	3	2	1	0	0	0	0
Catopsis	0	2	3	2	2	2	0
Glomeropitcairnia	1	0	0	0	0	0	0

hygrophilic flora such as Schimper's hypothesis calls for.

Table 12 lists the genera of the Tillandsioideae in order of increasing (taxonomic) "youth," that it is in order of increasing complexity of floral structure. It also gives for each genus the number of species that occur in the seven rain fall zones recognized in table 6 and described earlier. Table 12 is extracted from table 6. *Tillandsia,* with a naked polypetalous corolla and completely superior ovary, is both the most primitive and the most xerophytic genus; *Guzmania,* whose naked petals are connate, is less so. The concentration of *Vriesia* and *Thecophyllum* in the wetter parts of the island is very clear. All the species in both these genera have petal scales (nectaries) and a large number are truly gamopetalous (unpublished observations of the writer). *Glomeropitcairnia* has by far the most evolved floral structure in the subfamily: the petals bear nectaries and the ovary is half inferior. Also the seeds bear appendages at both ends. Its two species are known only from wet montane, or even cloud, forests in Trinidad, Venezuela, Martinique and Guadeloupe (Mez, 1935).

There are no easily accessible data on the subfamily elsewhere that could be reduced to the tabular form given here for the Trinidad species. The information that can be obtained from such works as the Pflanzenreich (Mez, 1935) are, however, in complete accordance with the Trinidad result that xerophytism in the Tillandsioideae is primary and the exploitation of humid epiphytic environments has been undertaken largely by the (taxonomically)younger genera.

(2) None of the tillandsioid species which were studied in Trinidad, representing every genus in the subfamily, gave any evidence of true shade-demand. They are all light demanding epiphytes, including the small percentage that do penetrate to some extent the shaded levels of forest. The latter are Shade-Tolerant rather than shade-demanding in the sense that they are maximally abundant in the highest available light intensities compatible with their high humidity requirements. Their exploitation of forest shade is clearly a secondary phenomenon associated with accommodation of the high humidity demands these species have acquired (cf. p. 83 above).

It should be noted here that Heinricher (1902, cited in Tietze, 1906) has found the seeds of most bromeliads incapable of germinating in the dark.

It is extremely difficult to reconcile either (1) the rigorous light demand that characterizes all the tillandsioids or (2) the strong xerophytism of the oldest genus with any theory that derives these epiphytes from a hygrophilic and shade-loving ground flora in wet forest.

(3) The essential feature in Mez's (1904) account of the physiology of the tillandsioid absorbing trichome is that it is a one-way valve permitting only the entrance of water. It is a most elaborate xerophytic adaptation and it is exceedingly difficult to understand its evolution in an environment such as the lower humid levels in rain forests where it has virtually no adaptive significance.

All three lines of evidence presented here clearly indicate that the Tillandsioideae entered forest primarily as light demanding xerophytes in the uppermost levels. Those species appearing now in lower forest levels have moved downwards; their status is secondary as shown by their residual light demand and the possession of a trichompomp.

The Bromelioideae. In the case of the Bromelioideae it is not possible to compare the relative xerophytism of "younger" and "older" genera since our incomplete knowledge of the corolla structure and the confused state of generic limits in this subfamily preclude any worthwhile judgment as to which are the "younger" genera. In any case only very few of the large number of Bromelioid genera are present in Trinidad. It is nevertheless significant that all the Trinidadian species are as light demanding as the tillandsioids, or more so.

Within the Bromelioideae there is another avenue of approach to the problem we are concerned with, since in this subfamily there are a great number of genera that contain only terrestrial plants completely dependent on the soil for nutrients and water. Intermediate stages in the evolution of the epiphytic habit can therefore be sought.

The two properties which have made the epiphytic existence possible in the Bromeliaceae are (1) the "tank-habit" of impounding a soil-equivalent in the form of water and humus between leaf bases, and (2) the system of leaf-epidermal absorbing trichomes which exploits this. The habit of *Bromelia humilis* (fig. 2) in Trinidad is the most direct evidence available in favor of the theory that these features evolved in semi-desert conditions. Thus *Bromelia humilis,* which is a member of a large genus that is exclusively and primitively terrestrial, shows the tank habit fully developed. The water, decaying leaves and the insect fauna between the leaves constitute a richer nutritive supply than the soil of the dry coastal desert scrub which it inhabits. This supply is exploited, as already described, by an upgrowing system of interfoliar (mycotrophic) roots; epidermal trichomes are abundant on the leaf bases protecting the roots from desiccation but they show little of the elaborate structure characteristic of the higher epiphytes. It seems very likely that the capacity of the epidermal trichomes to absorb not only water but minerals and amino-acids (Picado, 1913) would be acquired after rather than before their association with the rich interfoliar humus supply of a tank. It may be, in fact, that the tank habit exploited by a root system as in *B. humilis,* has actually been a precondition to the evolution in the family in general of the complete absorptive powers of the epidermal trichome. At the present time however there is no evidence for this other than the suggestive nature of the *B. humilis* adaptations. Be that as it may, this species is a concrete case of the development of the tank habit in response to a nutritionally poor xerophytic environment.

All the ecological evidence that is available from within the epiphytic subfamilies themselves, and independent therefore of their highly doubtful pitcairnioid ancestry, is in complete agreement with Tietze's main conclusion that the epiphytic Bromeliaceae have not evolved along the lines suggested by Schimper's hypothesis. The principal evolutionary trend in the Bromeliaceae has been towards nutritional independence of the substratum in response to dry impoverished soils. This independence, achieved through the tank habit and the absorbing trichome, has preadapted the higher sections of the family for epiphytic habitats and it is in these environments, secondarily invaded, that the Bromeliaceae have been most successful.

In its essentials this is doubtless the same pattern of exploiting ecological opportunity that has led to the epiphytic cacti such as *Epiphyllum, Rhipsalis,* and *Hylocereus.* However the bromeliads have been far more successful than the cacti as epiphytes, and this is undoubtedly associated with the fact that they were equipped to meet not only the water but also the nutritional problem in the forest canopy.

Summary

A classification of ecological types within the Bromeliaceae is developed. It is pointed out that the principal cleavage within the family is between species that are dependent on their substratum for nutrients and water and those that are not. Nutritive independence has been possible due to the evolution of (a) the "tank" habit of impounding water and humus between overlapping leaf bases, and (b) a system of epidermal absorbing trichomes on the leaves which exploit the water and humus of the tank.

The habit of *Bromelia humilis* Jacq. is described. The plant has a "tank" which it exploits by upgrowing interfoliar my-

cotrophic roots, a condition previously unknown in the family.

An analysis of the distribution of epiphytic bromeliads in varying conditions of light and humidity leads to the conclusion that light is their prime ecoclimatic variable.

All the ecological evidence is shown to be against Schimper's (1888) hypothesis, that epiphytes have evolved from the ground flora in wet forest, applying to the case of the Bromeliaceae. Epiphytism in the Bromeliaceae is held to be an exploitation of adaptive features developed initially in response to dry impoverished soils such as occur in semi-desert habitats.

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NOTES AND COMMENTS

THE CHROMOSOMES OF THE PARTHENOGENETIC MANTID *BRUNNERIA BOREALIS*

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Received November 19, 1947

Brunneria borealis Scudder (1896), a large, stick-like and almost apterous insect, is the only species of mantid which is known to reproduce exclusively by parthenogenesis. The evidence for this view has been summarized by Hebard (1942); although many juvenile and imaginal females have been collected from Texas, Oklahoma, Arkansas, Mississippi, Louisiana, Alabama, Florida, Georgia, South Carolina and North Carolina, not a single male has ever been found. The genus is one which has a wide distribution in South America, four species having been recorded from Patagonia, Uruguay, Paraguay, Bolivia and Brazil (Giglio-Tos, 1927). The South American species, however, are bisexual, males being apparently as common as females. *B. borealis* is restricted to the Southern states of the U. S. and has never been recorded from any of the West Indies or from Mexico; nor is any species of the genus known from the vast territory between Brazil and the Gulf Coast of the U. S. A. The published records would seem to indicate that *B. borealis* is locally common in East Texas, rather less so in the other Southern states, but this may merely indicate that collecting in Texas has been more intensive. The present work is based on material collected at Austin, Texas, in the fall of 1947. The species is not known to occur west of Austin, which is probably on the edge of its range (fig. 1).

The distribution of the genus *Brunneria* would seem to indicate that *B. borealis* reached its present area of distribution from the South.

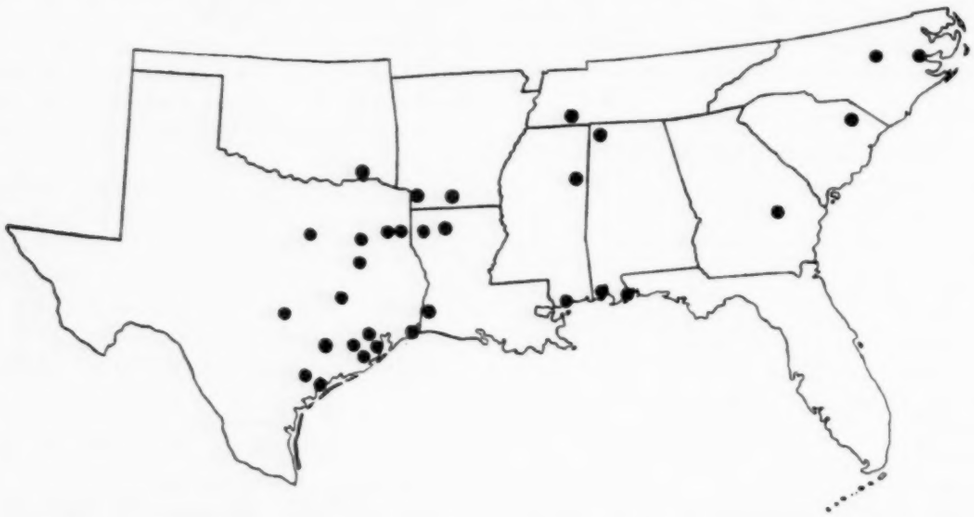

Fig. 1. Distribution of *Brunneria borealis*. Most of the records are from Hebard (1942); the following are unpublished: Alabama: between Pride and Barton, Colbert County (Rehn and Rehn)*; Arkansas: 7 miles east of Texarkana, Miller County (Rehn and Rehn)*; between Cargile and Upland, Union County (Rehn and Rehn)*; Mississippi: Columbus, Lowndes County (O. P. Breland—oothecae only); Pass Christian and Cat Island, Harrison County (Rehn and Hebard)*; Oklahoma: between Hugo and Grant, Choctaw County (Rehn and Rehn)*; S. Carolina: near Mars Bluff, Florence County (J. A. G. Rehn)*; Tennessee: near Guys, McNairy County (Rehn and Rehn)*. I am indebted to Mr. J. A. G. Rehn for the records marked with an asterisk, the specimens being in the series at the Academy of Natural Sciences, Philadelphia.

Possibly it was accidentally transported by sea or in a hurricane: it is entirely possible that the genus has never existed in Central America. We cannot tell whether the species was already parthenogenetic when it first established itself on the Gulf Coast, or whether it became so subsequently. Facultative parthenogenesis has been described in the African *Miomantis savignii* by Adair (1924) but it is not known how widespread it is in the group Mantoidea. Certainly, *Brunneria borealis* is the only species of mantid which is known to have entirely forsaken sexual reproduction.

Obligatory parthenogenesis is not very widespread in the other orthopterous groups of insects. In the Roaches the only well-authenticated case is in *Pycnoscelus surinamensis* L., where both bisexual and parthenogenetic strains exist (Matthey, 1945; Suomalainen, 1945*b*). In the Walking Sticks (Phasmoidea) some species of Bacillus, Leptynia, Eurycnema, Clonopsis and Carausius show obligatory parthenogenesis, but the vast majority of the genera contain only bisexual species. In the Saltatoria two instances of obligatory parthogenesis are known. The first is the little Cricket *Myrmecophila acervorum*, which lives in ants' nests in Southern Europe (Schimmer, 1909). The second is the large Tettigoniid *Saga pedo* Pallas, which ranges from the Balkans to Central Spain (other species of this genus, which occur in Palestine, Syria, Turkey and the Balkans, are bisexual). Occasional instances of facultative parthenogenesis have been recorded in various Gryllidae, Tettigoniidae and Acrididae (see Matthey, 1945 for details), but these occurred in species which are normally bisexual.

The only parthenogenetic Orthoptera which have been subjected to a really adequate cytological analysis are *Pycnoscelus surinamensis* and *Saga pedo*. The first of these is a diploid whose parthenogenesis is of the "ameiotic" type, there being two maturation divisions in the egg, both "equational" in type (Matthey, 1945). *Saga pedo*, on the other hand, is a tetraploid, having 68 chromosomes in its somatic nuclei (Matthey, 1941, 1946), the bisexual species *S. gracilipes* Uvar. and *S. ephippigera* Fisch. having 31 and 33 chromosomes in the somatic cells of the males (hence 32 and 34 in the females). *Saga pedo* is also ameiotic, but its eggs only undergo a single maturation division (equational in type). The cytological conditions in the parthenogenetic Phasmoidea apparently differ in the genera *Bacillus* and *Carausius*. *Bacillus rossii* is certainly a diploid (Von Baehr, 1907; Pehani, 1924; Cappe de Baillon, Favrelle and de Vichet, 1937; Cappe de Baillon and de Vichet, 1939); its parthenogenesis seems to be of the "meiotic" type, the somatic chromosome number being restored later, probably during the early cleavage divisions. There are two maturation divisions in the egg, 18 bivalents being visible in the first one. In *Carausius*, some species seem to have a chromosome cycle of the same type as that of *B. rossii*, while others show an ameiotic type of parthenogenesis with two "equational" maturation divisions in the egg. *C. theiseni* seems to be a diploid, while *C. furcillatus* and *C. morosus* are believed by Cappe de Baillon to be polyploid (triploids and tetraploids in different strains of *C. furcillatus*, possibly triploids in *C. morosus*).

At the end of September 1947 the author found a small colony of *Brunneria borealis* on a plot of waste land at Austin. The insects were resting on plants of *Aster exilis* Ell., whose flowers attracted a number of Bombyliids, on which the mantids were feeding. A few of the characteristic, very hard oothecae were collected at the same time, on stems of *Aster exilis* and on s' nted mesquite bushes growing nearby. In vi v of the previous cytological work on *Pycn scelus* and *Saga*, it seemed worth while to d rmine whether *B. borealis* is diploid or polyploid, it being well known that many parthenogenetic species of insects do exhibit polyploidy (Suomalainen, 1940, 1945*a*, 1947; Seiler, 1943). It would also be interesting to study the maturation divisions in the eggs of *B. borealis*, but this was not attempted with the present material.

The ovaries of six imagines were fixed in Flemming's fluid, embedded in paraffin, sectioned at 15 micra and stained in gentian violet by Newton's method. In order to facilitate the cutting of the sections the larger yolky oocytes were cut away before the material was embedded, only the smaller oocytes being utilised.

Somatic mitoses were numerous in the follicular nuclei surrounding the medium-sized oocytes. The metaphases were not particularly easy to study, owing to the fact that the chromosomes overlap considerably in most cells. In favorable cases, however, 28 chromosomes could be counted (fig. 2). Of these two are large metacentrics (V's) and evidently constitute a pair. The remaining chromosomes would probably be referred to as "rods" by most investi-

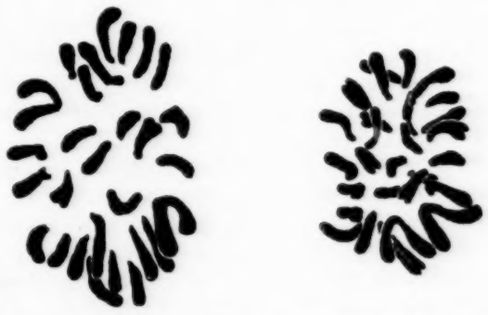

FIG. 2. Metaphase plates from follicle cells of medium-sized oocytes. 28 chromosomes in each.

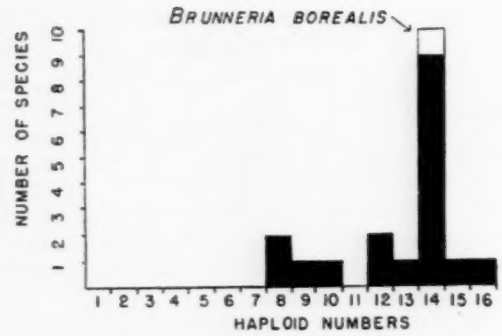

FIG. 3. Histogram showing the known chromosome numbers in the Mantoidea.

gators, but a careful study reveals that most of them have a "second arm" very much smaller than the main one and usually appearing as a little "satellite" on the centric end of the chromosome.

There can thus be no reasonable doubt that *B. borealis* is a diploid species. The fact that there are only two large metacentric chromosomes makes this certain and renders a comparison with the bisexual (and therefore necessarily diploid) species of the genus unnecessary. A glance at the histogram of the known chromosome numbers for the group Mantoidea (fig. 3) shows that 14 is the commonest haploid number for the bisexual species of mantids, so that a diploid number of 28 is by no means unexpected. The genus *Brunneria* has been placed by systematists in the subfamily Photininae. Only one member of this subfamily, the European *Iris oratoria,* has been studied cytologically (White, 1941); it has a haploid number of 13, i.e. one pair less than *B. borealis.*

In addition to the diploid nuclei, which are the commonest type in the follicular epithelium, a certain number were seen which showed about twice as many chromosomes. They are probably tetraploid, but owing to the great amount of crowding and overlapping of the chromosomes in the metaphase plates no exact counts were possible. The finding of a few tetraploid nuclei in an otherwise diploid epithelium is not unexpected and in no way affects the conclusion that *B. borealis* is a diploid species. It has long been known that in many species of insects a certain percentage of the follicle cells are tetraploid, presumably owing to failure of anaphase-separation at an earlier division (see Wilson, 1925, p. 871 for an instance of this in the Heteropteran *Anasa tristis*).

Brunneria borealis thus resembles the diploid parthenogenetic Orthoptera *Pycnoscelus surinamensis* and *Bacillus rossii* rather than the tetraploid *Saga pedo.*

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